

CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

**APPLICATION NUMBER
20-920**

Pharmacology Review(s)

Pharmacology/Toxicology Review and Evaluation**NDA number :** 20-920**Sponsor and/or agent:** Scios Inc.; Mountain View, CA**Division :** DCRDP; HFD-110**Center receipt date :** 1/10/01**Review receipt date :** 01/24/01**Drug : Trade name:** Natrecor®**Generic name :** Nesiritide (injection, 1.5 mg/vial)**Code name:** SC-70400**Chemical name:** Human B-Type Natriuretic peptide (hBNP₁₋₃₂)**Structure:** Natrecor® is a 32 amino acid peptide.

Molecular weight : 3464 g/mol (average mass of base form). A disulfide bridge connects the cysteine residues at positions 10 and 26, forming a ring of 17 amino acids with amino and carboxyl terminal extensions of 9 and 6 amino acids, respectively.

Drug class : Peptide hormone.**Indication :** Short-term intravenous treatment of congestive heart failure (CHF).**Route of administration :** Continuous intravenous infusion.

Formulation : Nesiritide ()mg (lyophilized), mannitol ()mg; Citric acid monohydrate ()mg; and Sodium citrate dihydrate ()mg.

Recommended dosage : 0.015 µg/kg/min.

Proposed studies : In response to the concerns raised by the Agency, this study was designed to assess whether tolerance develops to Natrecor as a result of prolonged infusion. A 72-hour continuous infusion of Natrecor in rabbits was proposed to assess blood pressure, which was found acceptable by the Agency.

Tolerance studies : Natrecor (0.1 µg/kg/min) was infused for 72 hours via the jugular vein of anesthetized New Zealand white rabbits. The carotid artery was used to monitor blood pressure and collect blood samples for cyclic GMP analysis at 3, 24, 48 and 72 hours.

In another study, rabbits were infused with Natrecor (0.1 µg/kg/min) for 72 hours and then norepinephrine (5 µg/kg/min) was infused to induce acute hypertension. After 30 min, rabbits were infused with Natrecor (0.5 µg/kg/min).

Results : Continuous IV infusion of Natrecor (0.1 µg/kg/min) for 72 hours resulted in a sustained elevation of plasma BNP levels by ~ 7-fold from baseline levels in rabbits. Plasma levels of cyclic GMP remained elevated by ~ 3.5-fold from basal levels during the 72 hours infusion period. In these normotensive rabbits, blood pressure tended to be reduced by ~ 12 mmHg during the first 3 hours of Natrecor infusion, but thereafter blood pressure returned to baseline levels. This is in contrast to the

previous report by the sponsor which showed that repeated bolus administration of h-BNP (3 µg/kg) every 60 min caused repetitive drop in systolic pressure by ~ 10 mmHg.

In the acute hypertensive rabbit model, norepinephrine infusion (5 µg/kg/min) resulted in an increase in systolic blood pressure by 15 - 20 mmHg in rabbits pretreated with Natreacor (0.1 µg/kg/min) for 72 hours. Thereafter, infusion of Natreacor (0.5 µg/kg/min) caused reversal of norepinephrine-induced increase in blood pressure towards baseline in ~ 45 minutes.

Summary : Based on the results of assays of cyclic GMP, which acts as a second messenger to cause vasodilation, there doesn't seem to be a substantial loss of activation of guanylate cyclase by infusing with Natreacor for 72 hours. This suggests lack of homologous desensitization. Similarly previous data submitted by the sponsor has shown that repeated bolus doses of hBNP caused increases in plasma cyclic GMP, urine volume and sodium excretion. In the current studies, there was a trend for reduction of blood pressure during the first 3 hours of Natreacor infusion, but not thereafter. Based on blood pressure as an end point, there seems to be loss of responsiveness to prolonged infusion with Natreacor.

In the acute hypertensive rabbit model induced by norepinephrine, the effectiveness of Natreacor to reverse the increase in blood pressure was intact in rabbits pretreated with Natreacor. In these studies, the dose of Natreacor (0.5 µg/kg/min) was increased by 5-fold to reverse norepinephrine-induced hypertension, whereas 0.1 µg/kg/min of Natreacor was used as a presumed desensitizing dosage. In these studies, it is not clear how increasing the dosage of Natreacor to overcome desensitization caused by a lower dose could answer the issue of tolerance. However, under these conditions infusion of Natreacor at 0.5 µg/kg/min antagonized the acute increase in blood pressure induced by norepinephrine in rabbits pretreated with Natreacor for 72 hrs prior to the norepinephrine challenge.

Conclusions : The lack of effect of Natreacor after 3 hours of infusion on blood pressure indicates that some degree of tolerance is evident after continuous exposure.

Issues : No reproductive toxicology studies were conducted with Natreacor. In a previous meeting (March 25, 1993), the need to perform reproductive toxicity for an endogenous peptide was regarded as not required.

No information is provided by the sponsor on carcinogenicity and genotoxicity studies such as chromosomal aberrations assay, mouse lymphoma cell assay, or in *in vivo* mouse micronucleus test. Previously the sponsor has shown data using strains of *Salmonella typhimurium* preincubated with Natreacor (1.79 mg/ml) which did not show increase in revertants suggesting no mutagenic potential.

/S/

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3 pages redacted from this section of
the approval package consisted of draft labeling

D. Willard

DEC 9 1998

NDA #20-920

NATRECOR[®]
(Nesiritide)

Scios, Inc.
Mountain View, California

Reviewer:
Thomas Papoian, Ph.D., D.A.B.T.
Pharmacologist

Division of Cardio-Renal Drug Products (HFD-110)
Center for Drug Evaluation and Research
Food and Drug Administration

December 8, 1998

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REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Thomas Papoian, Ph.D.
Dec. 8, 1998

1. INTRODUCTION:

SUBMISSION DATE: April 24, 1998
CENTER RECEIPT DATE: April 27, 1998
REVIEWER RECEIPT DATE: April 30, 1998

SPONSOR: Scios, Inc.,
Mountain View, California

DRUG:

Code Name: SC-70400

Generic Name: Nesiritide

Trade Name: Natrecor®

Chemical Name: Human B-Type Natriuretic Peptide (hBNP₁₋₃₂)

CAS Registry Number: None

Structure:

Natrecor® is a 32-amino-acid peptide with the following amino acid sequence:

SerProLysMetValGlnGlySerGlyCysPheGlyArgLysMetAspArgIleSerSerSerSerGlyLeuGlyCysLysValLeuArgArgHis
1 5 10 15 20 25 30 32

Empirical Formula: C₁₄₃H₂₄₄N₅₀O₄₂S₄

Molecular Weight: 3464 g/mol (average mass of base form)

A disulfide bridge connects the cysteines at positions 10 and 26, forming a ring of 17-amino acids with amino and carboxyl terminal extensions of 9 and 6 amino acids, respectively.

RELATED INDs/NDAs/DMFs: IND^F

PHARMACOLOGIC CLASS: Peptide hormone

PROPOSED CLINICAL INDICATION: For the short-term intravenous therapy of congestive heart failure (CHF).

FORMULATION: - Nesiritide, () mg (lyophilized), mannitol, () mg, citric acid monohydrate () mg, and sodium citrate dihydrate, () mg.

ROUTE OF ADMINISTRATION: Continuous intravenous infusion

RECOMMENDED DOSAGE: 0.015 µg/kg/min

RATIONALE: Human B- type natriuretic peptide (hBNP; previously known as human brain natriuretic peptide) is a 32- amino acid peptide which is a naturally occurring cardiac hormone produced primarily in the cardiac ventricle and which has vasodilatory, diuretic, natriuretic, and neurohormonal effects. Natrecor (nesiritide) is a preparation containing the purified peptide produced by recombinant DNA technology, with an amino acid sequence identical to the endogenous hBNP cardiac hormone.

Endogenous levels of hBNP are elevated in patients with systolic and diastolic cardiac dysfunction and cardiac hypertrophy. Human BNP binds to the particulate guanylate cyclase receptor (GC- A) of vascular smooth muscle and endothelial cells, leading to increased intracellular concentrations of guanosine 3'5'- cyclic monophosphate (cGMP) which acts as a second messenger to cause smooth muscle cell relaxation and subsequent vasodilation. Venodilation promotes peripheral pooling of blood and decreases venous return to the heart, thereby reducing left ventricular end diastolic pressure and pulmonary capillary wedge pressure (PCWP) (preload). Arterial dilation reduces systemic vascular resistance (afterload) and systemic and pulmonary arterial pressure. Human BNP has also been shown to reduce plasma concentrations of aldosterone in patients and to have mild diuretic and natriuretic properties.

In subjects with acutely decompensated congestive heart failure, nesiritide produces, according to the sponsor, dose-related improvements in clinically relevant hemodynamic measures, including reductions in pulmonary capillary wedge pressure, mean right atrial pressure, and systemic vascular resistance, and increases in cardiac index and stroke volume index, without a significant effect on heart rate or cardiac work. These are said to lead to improvements in specific symptoms of decompensated CHF, such as dyspnea, fatigue, and peripheral edema.

2. PHARMACOLOGY:

2.1. Activation of cellular guanylyl cyclase by human brain natriuretic peptide (Report No. 93-017-70400; Vol. 13 pp. 96-99):

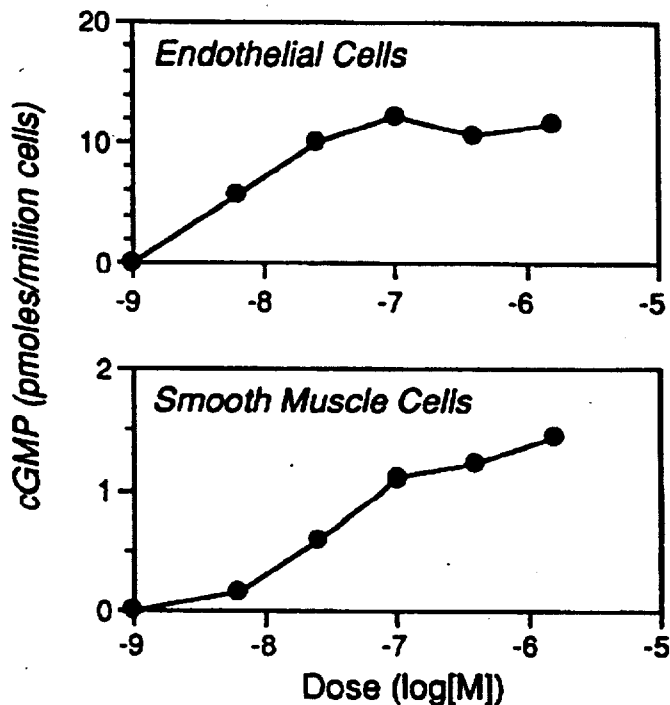
Purpose: This study was designed to measure and compare the induction of cGMP production in response to hBNP in primary bovine vascular smooth muscle cells and primary bovine endothelial cells.

Methods: Primary cultures of bovine aortic smooth muscle (BASM) cells and bovine aortic endothelial (BAE) cells were incubated with hBNP at 1.5×10^{-10} to 1.6×10^{-6} M for 2 min at 37° C. Amounts of cGMP were measured by radioimmunoassay.

Results: hBNP stimulated cGMP in a dose-dependent manner. For BAE cells the $EC_{50} = 9.0 \times 10^{-7}$ M. For BASM cells the $EC_{50} = 1.7 \times 10^{-8}$ M. The magnitude of the cGMP response to hBNP in BAE cells was about 10X higher than in BASM cells (Figure 1; Sponsor's Figure 1).

Figure 1 (Sponsor's Figure 1)

Effect of hBNP on cGMP Production in Bovine Aortic Endothelial Cells and Bovine Aortic Smooth Muscle Cells *In Vitro*



Conclusions: It was concluded by the Sponsor that the hBNP-mediated increase in cGMP in these cells was due to activation of the guanylyl cyclase A (GC-A) cell surface receptor, and that cGMP was likely to be the second messenger mediating most of hBNP's action of vasodilation *in vivo*. This mechanism is similar to nitric oxide's vasodilatory effect in which the nitric oxide-mediated activation of guanylate cyclase and accumulation of cGMP has been shown to be associated with activation of the cGMP-dependent protein kinase. This kinase dephosphorylates specific smooth muscle cell proteins and results in vessel relaxation.

2.2. Pharmacokinetics and induction of plasma cyclic GMP following intravenous bolus and continuous infusion of recombinant and synthetic hBNP (Report No. 00181; Vol. 13 pp. 100-121):

Purpose: Previous studies in humans and dogs have shown that the intracellular accumulation of cGMP that occurs after activation of the guanylyl cyclase receptor by hBNP results in release of cGMP from the cell into the circulation. The present studies measured

elevations in plasma cGMP in rabbits after administration of either synthetic (syn) or recombinant (rec) hBNP by either bolus i.v. injection or continuous i.v. infusion. Pharmacokinetics were also measured.

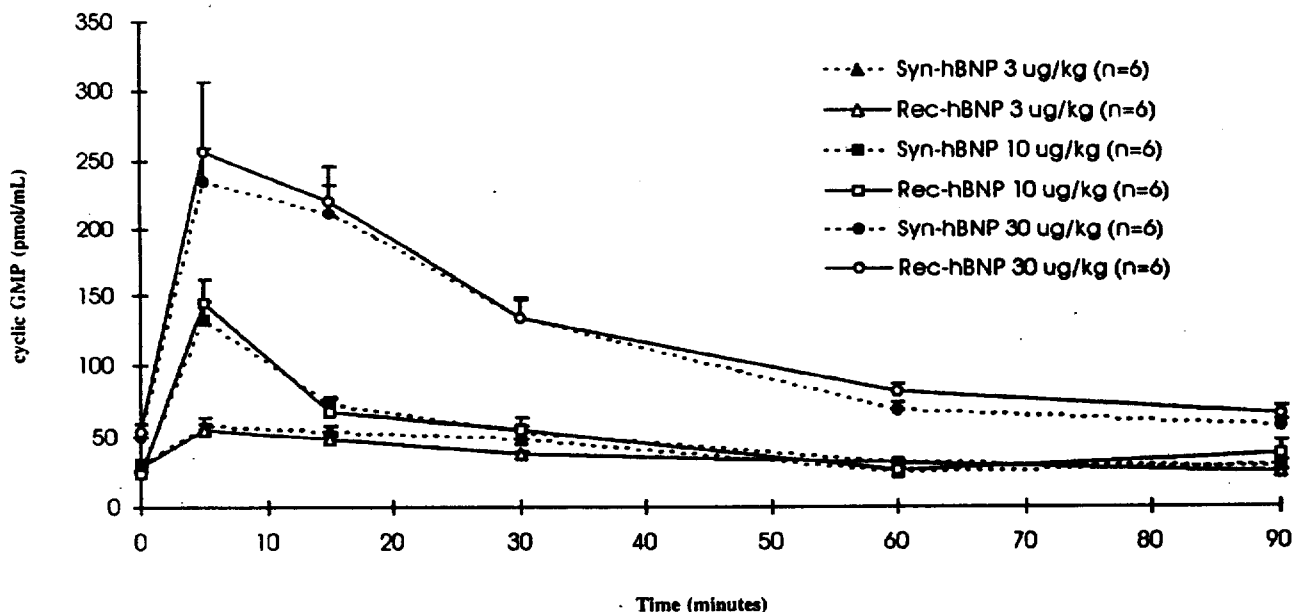
Methods: Male New Zealand White rabbits (2.5-3.0 kg) were used in two separate studies. In the first study, rabbits (6/group) were given either syn-hBNP or rec-hBNP at 3, 10, or 30 $\mu\text{g/kg}$ by bolus i.v. administration. The actual values for syn-hBNP (76.2% net peptide) were 2.3, 7.6, or 22.8 $\mu\text{g/kg}$, and for rec-hBNP (97% net peptide) the actual values were 2.9, 9.7, or 29 $\mu\text{g/kg}$. Blood was withdrawn for up to 90 min for determination of cGMP levels by radioimmunoassay and hBNP levels by an antigen displacement assay (ELIZA).

In the second study, rabbits (7/group) received escalating doses of either syn-hBNP or rec-hBNP at 50, 100, and 200 ng/kg/min of drug substance for 60 min for each dose by intravenous continuous infusion. Blood was taken at the end of infusion (50-60 min, 110-120 min, and 170-180 min for the 50, 100, and 200 ng/kg/min doses, respectively) for determination of cGMP and hBNP levels.

Results: In the first study, bolus administration resulted in a time- and dose-dependent increase in plasma cGMP levels (Figure 2; Sponsor's Figure 1). There were essentially no differences in plasma cGMP levels after either syn-hBNP or rec-hBNP administration.

Figure 2 (Sponsor's Figure 1)

Effect of rec-hBNP and syn-hBNP on Plasma cyclic GMP in Normotensive Conscious Rabbits

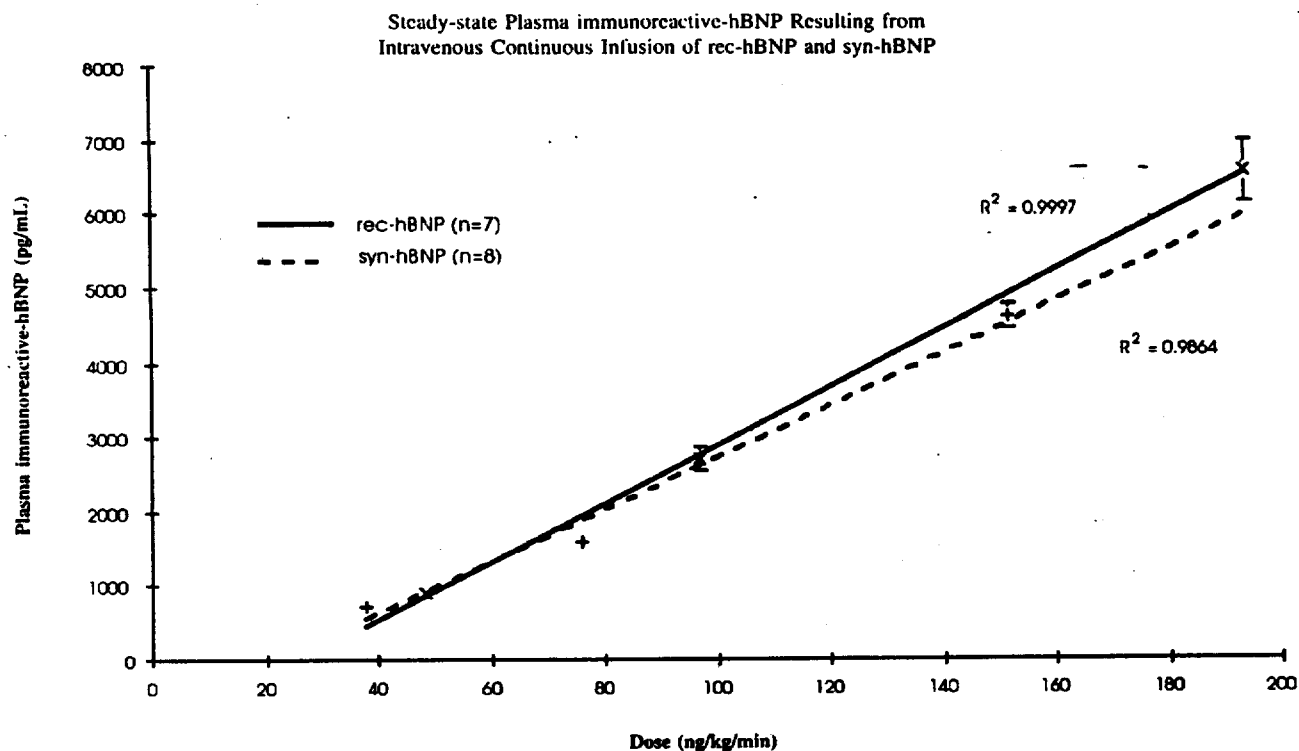


An approximate half-life for plasma cGMP was derived to be 20.6 ± 5.9 min. Plasma levels after the 30 $\mu\text{g/kg}$ dose were best fit to a two compartment model that predicts drug concentrations declining biexponentially as the sum of two first-order processes. Plasma levels after the 10

$\mu\text{g/kg}$ dose were best fit to a one compartment model that predicts drug concentrations declining exponentially in the first-order process. There were essentially no differences in pharmacokinetic parameters for syn-hBNP or rec-hBNP after i.v. bolus administration.

In the second study, there was an approximately a 5-7-fold increase in plasma cGMP over baseline after administration of either syn-hBNP or rec-hBNP by continuous infusion. The relationship between plasma concentration and infusion rate during continuous infusion of syn-hBNP and rec-hBNP was similar (Figure 3; Sponsor's Figure 5). There were essentially no differences in pharmacokinetic parameters for syn-hBNP or rec-hBNP after i.v. continuous infusion.

Figure 3 (Sponsor's Figure 5)



Conclusions: Plasma cGMP levels were used as an *in vivo* marker of hBNP receptor activation which leads to vasodilation through the activation of the cGMP-dependent protein kinase. After bolus administration, plasma cGMP levels increased in a time- and dose-dependent manner. There were no significant differences in the cGMP response or in pharmacokinetic parameters after bolus or continuous infusion of either syn-hBNP or rec-hBNP. In rabbits given the high dose of 30 $\mu\text{g/kg}$ syn-hBNP or rec-hBNP, a rapidly metabolized α phase and a more slowly metabolized β phase were detected when compared to the lower dose of 10 $\mu\text{g/kg}$. There was no difference in the steady-state metabolic clearance rates derived from intravenous continuous infusion of syn-hBNP and rec-hBNP.

2.3. Vascular relaxant effect of human B-type natriuretic peptide (hBNP) on isolated human arterial and venous tissue (Study No. 00256; Vol. 13 pp. 122-139):

Purpose: This study tested whether hBNP relaxes isolated human arterial and venous tissue preparations that were precontracted with either endothelin-1 (ET) or the alpha adrenergic agonist phenylephrine (PE). The arterial relaxant effects of ANP were compared to those of hBNP. Human tissue was used because previous studies have shown a weak relaxant effect of hBNP on rat vascular tissue.

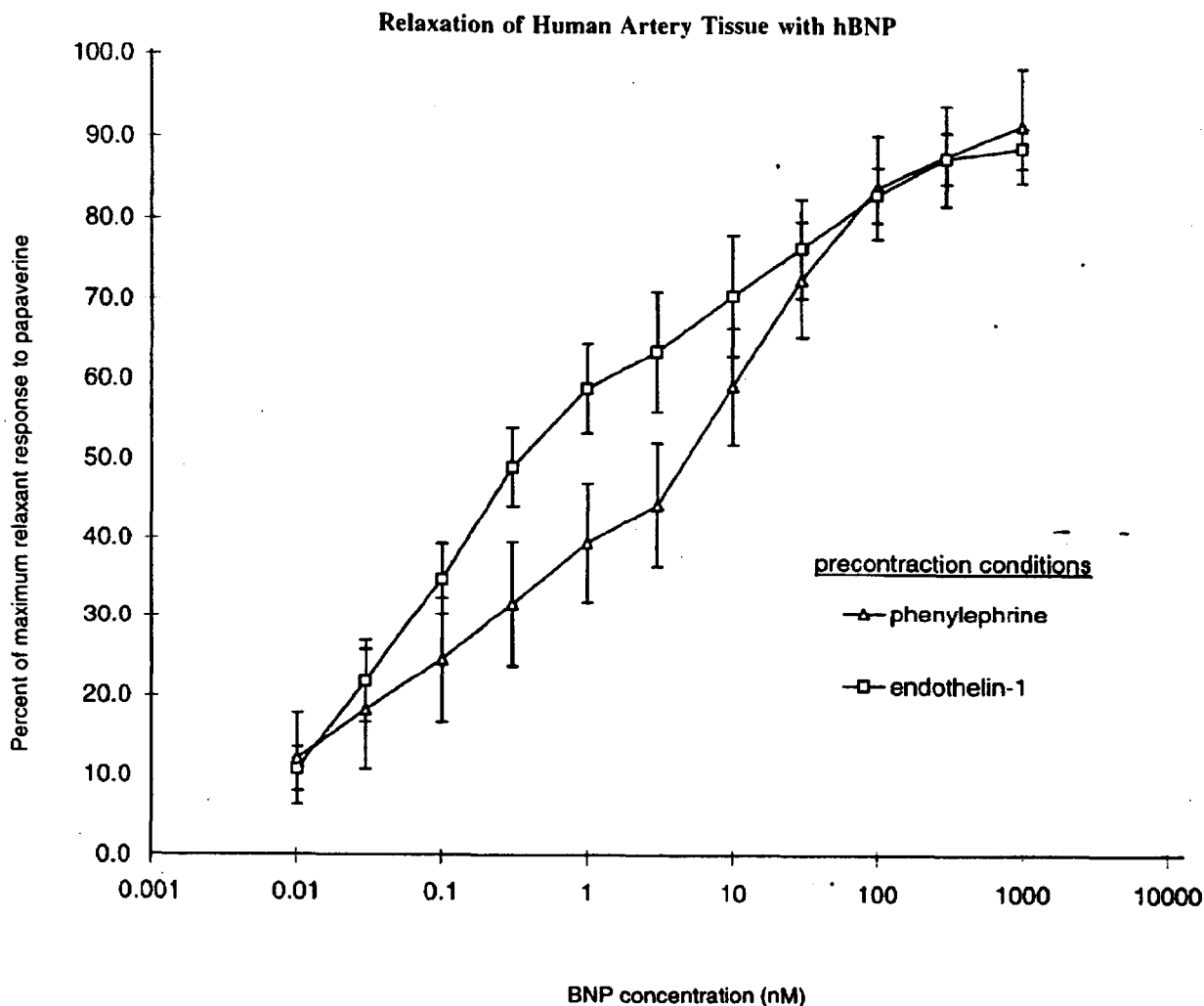
Methods: Samples of internal mammary artery and saphenous vein were obtained from patients undergoing various types of cardiac surgery, primarily coronary bypass surgery and valve replacement. Rings were cut 3 mm in diameter and attached to a force-displacement transducer for measuring tension development. Rings were contracted with ET or PE to 70% of the maximal response measured with KCl. Increasing concentrations of hBNP or ANP were added and the relaxant response measured. After the response had reached a maximum, papaverine was added to the bath to determine the maximum relaxant response. The response to each concentration of test compound evaluated was normalized as a percent of the maximum relaxant response obtained to papaverine.

Results: As shown in Figure 4 (Sponsor's Figure 1), hBNP induced a concentration-dependent relaxant effect on arterial vascular tissue precontracted with ET and PE ($EC_{50} = 1.9 \pm 1.5$ nM and 10 ± 4 nM, respectively). A statistically significant relaxant response to hBNP was seen at concentrations ≥ 0.03 nM for arterial vascular tissue precontracted with both ET and PE.

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Figure 4 (Sponsor's Figure 1)

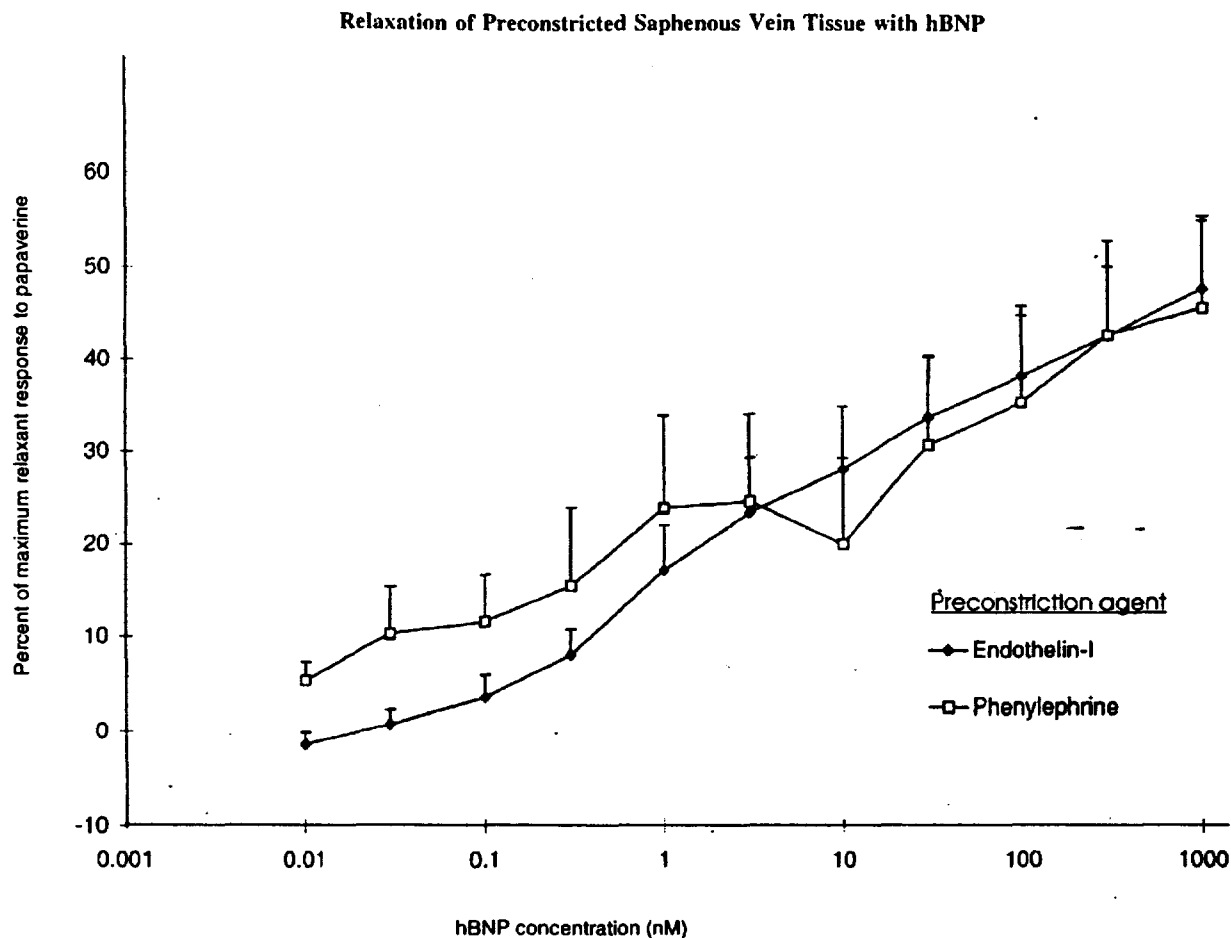


The effect of ANP on arterial vascular tissue precontracted with ET and PE was similar to that seen with hBNP ($EC_{50} = 1.8 \pm 1.0$ nM and 19 ± 7 nM, respectively). A statistically significant relaxant response to ANP was seen at concentrations ≥ 0.03 nM and ≥ 1.0 nM for arterial vascular tissue precontracted with ET and PE, respectively.

On saphenous vein tissue precontracted with ET or PE, hBNP induced a concentration-dependent relaxant effect that was statistically significant at concentrations ≥ 1.0 nM (Figure 5; Sponsor's Figure 3).

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Figure 5 (Sponsor's Figure 3)



Conclusions: hBNP showed relaxant activity in precontracted cultures of human arterial and venous tissue. This effect was concentration-dependent and was similar to that seen with ANP. hBNP induced statistically significant responses on arterial tissue at ≥ 0.03 nM and on venous tissue at ≥ 1.0 nM. The Sponsor concluded that the therapeutic concentration for hBNP would be about 1 nM, and that this concentration *in vivo* would be expected to exert a vasodilatory effect.

2.4. Coronary vasodilator effects of human B-type natriuretic peptide: Mechanisms of action in coronary conductance and resistance arteries (Study No. 00275; Vol. 13 pp. 140-164):

Purpose: This study examined the mechanisms involved in the coronary vasodilatory response to recombinant hBNP in anesthetized pigs, specifically the effects of hBNP on coronary conductance and resistance arteries under conditions of both normal vascular tone and endothelin-1 (ET-1)-induced pre-constriction. Also studied were the possible contributions of nitric oxide, prostaglandins, and ATP sensitive potassium channels to hBNP-induced coronary vasodilation.

Methods: Female domestic swine were anesthetized with Innovar and alpha-chloralose. The left coronary artery was cannulated with a Doppler wire and then with an ultrasound imaging catheter for determination of luminal cross-sectional areas (CSA) and coronary blood flow (CBF) velocities. Transvenous atrial pacing was used to prevent changes in heart rate.

hBNP was infused at concentrations increasing from 1 pM to 0.1 μ M. Nitroglycerin (positive control) was infused at concentrations increasing from 0.1 nM to 10 μ M. Epicardial coronary dimensions and flow velocity were allowed to return to baseline before the next dose was given (range 5-9 min). The vasodilator effect of hBNP was also assessed following precontraction with 10 nM endothelin-1. Additional pigs were used to assess the effects of pharmacologically active doses of hBNP (10 nM to 0.1 μ M) after the following pharmacological interventions:

- (1) inhibition of nitric oxide synthesis by intracoronary administration of nitro-L-arginine methylester (L-NAME);
- (2) inhibition of prostaglandin synthesis by intravenous infusion of indomethacin; and
- (3) inhibition of ATP-sensitive potassium channels by intracoronary administration of glibenclamide.

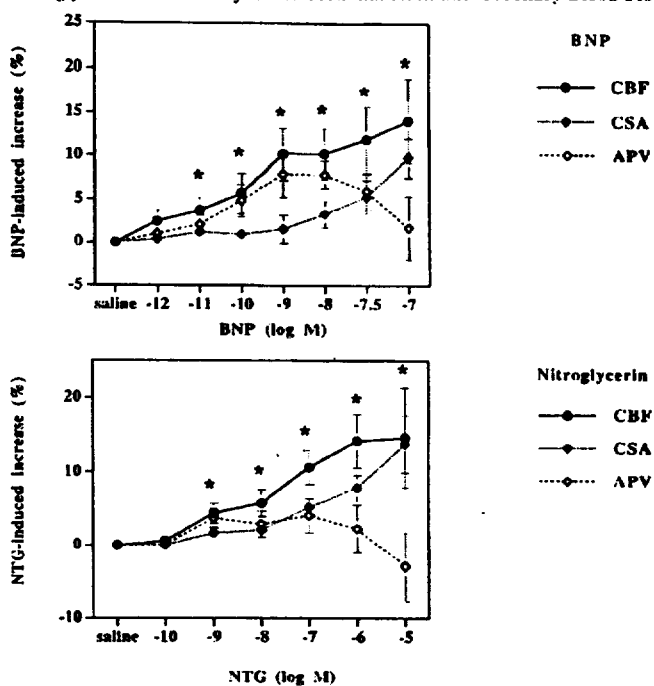
[Note: Effects of the above inhibitors on baseline parameters (CSA, APV, CBF) in the absence of hBNP were not measured. Results of inhibitor effects were expressed as percent change from hBNP treatment alone.]

Results: hBNP caused significant dose-related increases in luminal cross-sectional areas (CSA), and coronary blood flow (CBF) velocities (Figure 6; Sponsor's Figure 1). At lower doses, blood flow increased without an increase in luminal area (CSA) suggesting vasodilation of resistance vessels. Luminal area of the coronary vessel increased at the higher doses. Average peak velocities (APV) increased at the intermediate doses, but then returned to baseline values at the higher doses. There were no significant changes in systemic arterial pressure or heart rate at any of the doses tested. Effects of nitroglycerin (NTG) were similar to those of hBNP (Figure 6; Sponsor's Figure 1).

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Figure 6; Sponsor's Figure 1

Effects of Increasing Concentrations of hBNP and
Nitroglycerin on Coronary Cross-Sectional Area and Coronary Blood Flow

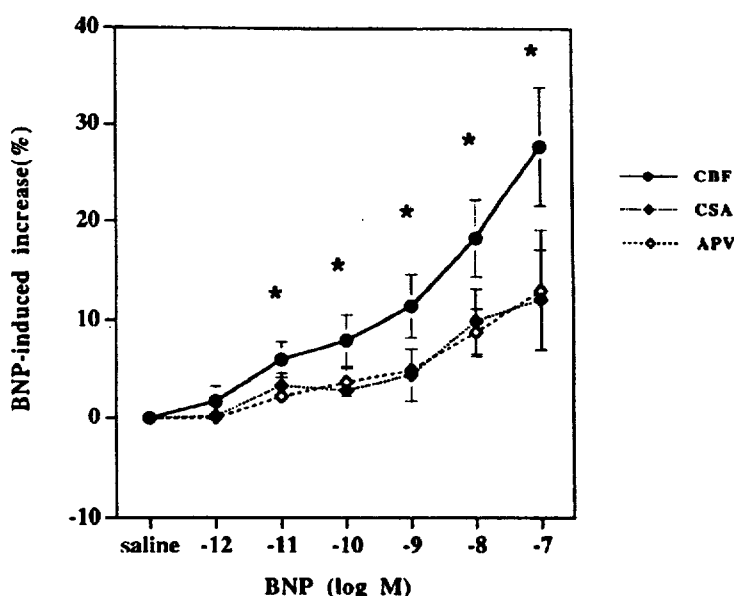


In vessels precontracted with ET-1, hBNP increased luminal area (CSA), average peak velocity (APV), and blood flow (CBF) in a dose-dependent manner (Figure 7; Sponsor's Figure 2).

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Figure 7 (Sponsor's Figure 2)

Effects of hBNP on CBF and Coronary CSA
in Coronary Arteries Pre-Constricted with Endothelin-1



Inhibition of nitric oxide synthesis by intracoronary administration of nitro-L-arginine methylester (L-NAME) did not significantly change the vasodilatory response (increased luminal area) to hBNP, indicating that the hBNP-induced vasodilation in conductance vessels was not dependent on nitric oxide synthesis. However, L-NAME did significantly attenuate the hBNP-induced increases in velocity (APV) and blood flow (CBF) indicating that these effects of hBNP in resistance vessels were mediated through synthesis of nitric oxide.

Inhibition of prostaglandin synthesis by intravenous infusion of indomethacin reduced the hBNP-induced increases in luminal area (CSA), velocity (APV), and blood flow (CBF), although only APV and CBF responses to hBNP were significantly attenuated by indomethacin. This indicated that the vasodilatory effects of hBNP may be dependent upon prostaglandin synthesis.

Inhibition of ATP-sensitive potassium channels by intracoronary administration of glibenclamide did not attenuate, but rather increased, the vasodilatory response (luminal area; CSA) induced by hBNP. However, glibenclamide did not alter velocity (APV) or blood flow (CBF) induced by hBNP. This indicated that the vasodilatory effects of hBNP were not dependent upon ATP-sensitive potassium channels that normally help regulate coronary vascular tone.

Conclusions: hBNP was shown to exert vasodilatory effects *in vivo*. Coronary blood flow increased in the absence of increases in blood pressure or heart rate. The hBNP-induced increases in vasodilation and coronary blood flow were similar to those seen with nitroglycerin. The vasodilatory effect of hBNP after precontraction with endothelin-1 suggested to the sponsor a possible treatment during coronary spasm-induced angina or ischemia. Endothelium-derived nitric oxide contributes to hBNP-induced vasodilation in resistance vessels but not in

conductance vessels. The vasodilatory effects of hBNP may be dependent upon prostaglandin synthesis and release. The vasodilatory effects of hBNP were not dependent upon ATP-sensitive potassium channels that normally help regulate coronary vascular tone.

The Sponsor speculated that some of the effects of hBNP seen in human patients may in fact be due to increased levels of ANP, since administered hBNP may compete with ANP for metabolism by the natriuretic peptide clearance receptor or the neutral endopeptidase resulting in higher levels of ANP.

2.5. Cardiovascular and renal response of rabbits to Natrecor hBNP (Study No. 00107; Vol. 13 pp. 164-180):

Purpose: Published studies have shown that human BNP (hBNP) is about 10X less potent than rat BNP in inducing cGMP in rat vascular smooth muscle cells. hBNP appears to be more effective in dogs and baboons than in rats. Therefore, the rabbit was studied as for its suitability as a model for hBNP action. Hemodynamic (mean, systolic, diastolic, and pulse pressures), cardiac (heart rate), hormonal (urine cyclic GMP), and renal (urine volume, urine sodium, urine potassium) responses were measured.

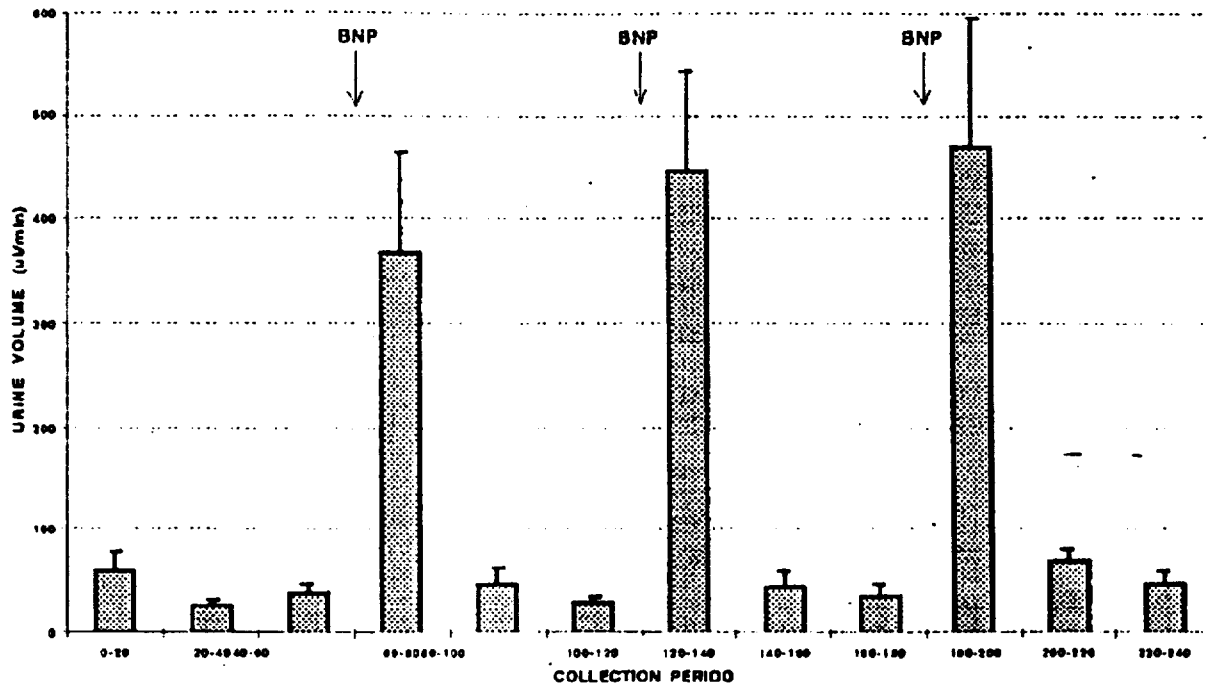
Methods: Male New Zealand White rabbits were anesthetized with pentobarbital and catheterized for analysis of cardiovascular responses (systolic, diastolic, and mean arterial pressures, heart rate). Urine was also collected for determination of urine volume and for sodium and potassium. Urine cGMP was measured using an enzyme-linked immunoassay.

The experimental protocol was divided into four 60 min periods. Sixty min after an initial bolus injection of vehicle (saline), a bolus dose of hBNP (3 µg/kg) was given 3X with a 60 min interval between doses. Cardiovascular data was monitored continuously, and urine was collected at 20 min intervals. Control rabbits received 4 bolus injections of saline at 60 min intervals.

Results: Renal effects: During the first 20 min collection periods following the first, second, and third doses of hBNP, urine volume increased 9-11X over baseline with similar increases between the first and third challenge (Figure 8; Sponsor's Figure 1). Urine volume returned to near baseline by the second 20 min collection period for each dose given. Urine sodium (natriuresis) increased 11-13X over baseline with similar increases between the first and third challenge (Figure 9; Sponsor's Figure 2). Urine sodium returned to near baseline by the second 20 min collection period for each dose given. Urine potassium values were below the limit of detection making an accurate assessment difficult. Urinary cGMP values showed larger variations after dosing, but were generally increased ~6-10X within 20 min after dosing. cGMP values did not return to baseline before the next dose was given resulting in slightly higher cGMP levels with each subsequent dose.

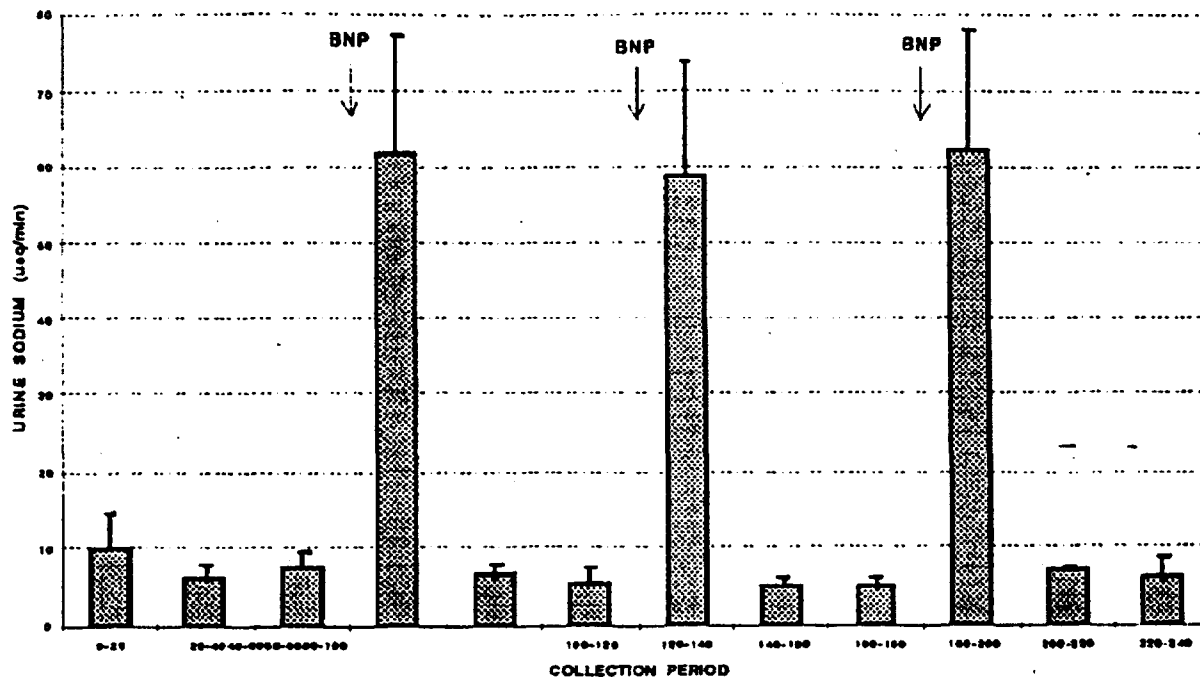
Figure 8 (Sponsor's Figure 1)

Effect of hBNP on Urine Volume in Rabbits



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Figure 9 (Sponsor's Figure 2)

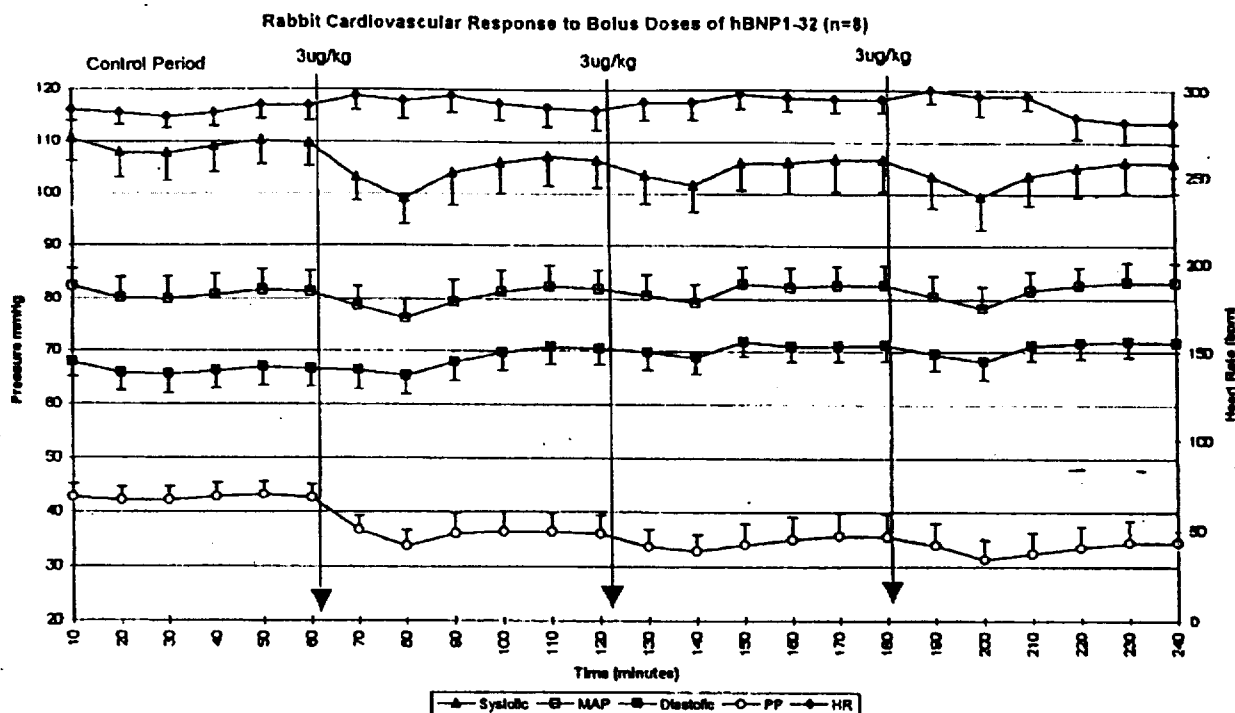
Effect of hBNP on Urine Sodium in Rabbits

Cardiovascular effects: hBNP administration caused a 10 mm Hg drop in systolic blood pressure without significantly affecting diastolic blood pressure (Figure 10; Sponsor's Figure 5). The hypotension reached its nadir about 20 min after each administration, but returned to near baseline levels by 60 min. Reduced pulse pressure, a reflection of decreased venous return, was present after the first dose and remained lower throughout the experimental period.

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Figure 10 (Sponsor's Figure 5)

Cardiovascular Effects of hBNP in Rabbits



Conclusions: Bolus administration of 3 µg/kg hBNP had significant, but transient, diuretic (9-11X increase in urine volume) and natriuretic (11-13X increase in sodium excretion) effects in rabbits. Repeated administration of hBNP 60 min apart did not result in desensitization of the effects. There was a corresponding drop in systolic blood pressure that correlated with the renal diuretic and natriuretic effects. The hypotension reached its nadir about 20 min after each administration, but returned to near baseline levels by 60 min. The Sponsor stated that the effects seen in the rabbit were similar to those seen in dogs, and that the rabbit appeared to be a suitable model to study the pharmacologic actions of hBNP.

2.6. Renal and hemodynamic effects of recombinant and synthetic hBNP in anesthetized rabbits (Study No. 00159; Vol. 13 pp. 181-195):

Purpose: Based on the results seen in the previous study in which hBNP increased urine volume and sodium excretion, and reduced systolic blood pressure in rabbits, the present study used these same endpoints to compare the activities of synthetic (syn) versus recombinant (rec) hBNP in rabbits.

Methods: Male New Zealand White rabbits were anesthetized with pentobarbital and catheterized for analysis of cardiovascular responses (systolic, diastolic, and mean arterial

pressures, heart rate). Urine was also collected with a catheter for determination of urine volume and for sodium and potassium.

The treatment protocol was divided into four 60 min periods. Sixty min after an initial bolus injection of vehicle (saline), a bolus dose of either syn-hBNP or rec-hBNP at 1, 3, or 10 µg/kg was given 3X with a 60 min interval between doses (Note: The percent of net peptide in the rec-hBNP and syn-hBNP drug substance preparations used were 97% and 76.2%, respectively. Thus, there was approximately 22% less syn-hBNP peptide than rec-hBNP peptide). Cardiovascular data was monitored continuously, and urine was collected at 20 min intervals. Control rabbits received 4 bolus injections of saline at 60 min intervals.

Results: Renal effects: Increasing doses of either syn-hBNP or rec-hBNP produced transient increases in urine volume and sodium excretion. The diuretic and natriuretic effects occurred within the first 20 min after dosing and returned to near baseline levels by 60 min. The increase in urine volume was significant at 3 µg/kg, while the increase in sodium excretion was significant at 1 µg/kg. There were no significant increases in potassium excretion when compared to the saline controls. There were essentially no differences between the synthetic or recombinant forms of hBNP.

Cardiovascular effects: Increasing doses of either syn-hBNP or rec-hBNP produced transient decreases in systolic blood pressure, pulse pressure, and mean arterial pressure that were statistically significant at 3 µg/kg. Similar to the renal effects, the cardiovascular effects occurred within the first 20 min after dosing and returned to near baseline levels by 60 min. Again, there were essentially no differences between the synthetic or recombinant forms of hBNP.

Conclusions: Intravenous bolus administration of hBNP, in either the synthetic or recombinant form, resulted in significant, but transient, diuresis (≥ 3 µg/kg) and natriuresis (≥ 1 µg/kg) without significant kaliuresis in rabbits (up to 10 µg/kg). Systolic blood pressure, pulse pressure, and mean arterial pressure were decreased (≥ 3 µg/kg) and these cardiovascular effects correlated with the renal effects, in that the effects occurred within the first 20 min after dosing and returned to near baseline levels by 60 min. There were no significant differences in renal or cardiovascular effects in response to syn-hBNP versus rec-hBNP administration in rabbits.

2.7 Natrekor hBNP reduces acute hypertension induced by norepinephrine in anesthetized rabbits (Study No. 00120; Vol. 13 pp. 196-208):

Purpose: Previous studies showed that hBNP relaxed human artery tissue that had been precontracted with the alpha adrenergic receptor agonist phenylephrine. The present study measured the effect of hBNP on acute hypertension induced by norepinephrine in rabbits.

Methods: Male New Zealand White rabbits were anesthetized with pentobarbital. The femoral artery was catheterized for collection of cardiovascular data including heart rate, systolic blood pressure, and diastolic blood pressure. Norepinephrine was given i.v. at 5 µg/kg/min for the duration of the experiment. Fifteen minutes after the start of the norepinephrine infusion, a bolus infusion of recombinant hBNP was given at 30 µg/kg. Controls received saline. Blood pressure and heart rates were monitored for 90 min.

Results: In rabbits given norepinephrine the systolic blood pressure rose a maximum of 35-40 mm Hg 5 min after administration and declined gradually to about 20 mm Hg above baseline after 80 min. Diastolic and mean arterial blood pressures were similarly affected after norepinephrine administration. Norepinephrine reduced heart rate 25 bpm initially, but then the heart rate returned to baseline after 60 min. Administration of hBNP reduced the rise in systolic blood pressure from about 35-40 mm Hg to about a 10 mm Hg rise which was maintained throughout the infusion period. The norepinephrine-induced rise in diastolic and mean arterial blood pressure were similarly reduced by hBNP. hBNP had no effect on the norepinephrine-induced drop in heart rate. In control rabbits saline had no effect on the norepinephrine-induced changes in blood pressure or heart rate.

Conclusions: hBNP reduced the norepinephrine-induced increase in blood pressure (acute hypertension) in rabbits, while it had no effect on the norepinephrine-induced drop in heart rate. The Sponsor suggested that hBNP may be useful in controlling blood pressure in the setting of post-surgical hypertension.

2.8. Hemodynamic effects of continuous infusion of human B-type natriuretic peptide (hBNP) in normotensive conscious rabbits and conscious rabbits with acute norepinephrine-induced hypertension (Study No. 00238; Vol. 13 pp. 209-231):

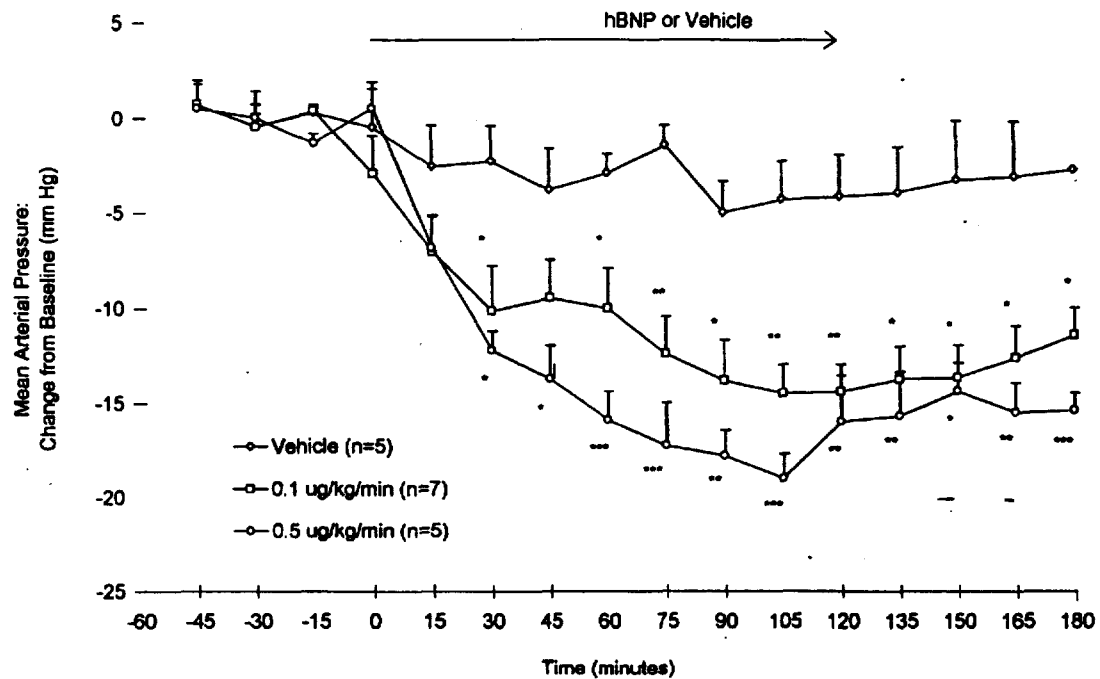
Purpose: An association has been reported between elevated plasma catecholamines, plasma dopamine beta hydroxylase activity, and acute hypertension following surgery. This study further explored the hemodynamic effects of hBNP in rabbits with norepinephrine-induced hypertension as a model for examining the use of hBNP for controlling acute hypertension associated with surgical procedures.

Methods: Conscious male New Zealand White rabbits were catheterized in the ear artery for blood pressure measurements (systolic, diastolic, and mean arterial pressures). Recombinant hBNP was given i.v. into the ear vein at either 0.1 or 0.5 $\mu\text{g/kg/min}$ for 2 hours. Controls received saline. Some of the rabbits were infused with norepinephrine at 5 $\mu\text{g/kg/min}$ for one hour before the administration of hBNP to contract the vessels and induce hypertension. Norepinephrine was continued for an additional 2 hours (3 hours total). All rabbits were monitored throughout the infusion periods.

Results: Treatment with hBNP in normotensive rabbits significantly reduced blood pressure by 30 min which reached a nadir by 105 min (Figure 11; Sponsor's Figure 3). Intravenous infusion of 0.5 $\mu\text{g/kg/min}$ of hBNP decreased systolic, diastolic, and mean arterial pressures by 21%, 18%, and 19%, respectively. Blood pressure at the 0.5 $\mu\text{g/kg/min}$ dose appeared to rise during the last 15 min of infusion. At a dose of 0.1 $\mu\text{g/kg/min}$, hBNP decreased systolic, diastolic, and mean arterial pressures by 15%, 14%, and 14%, respectively.

Figure 11 (Sponsor's Figure 3)

Effect of Continuous Intravenous Infusion of hBNP on Mean Arterial Pressure in Conscious Normotensive Rabbits



*P < 0.05, **P < 0.01, ***P < 0.001 for comparison of values at each timepoint versus baseline (t=0) with Dunnett multiple comparison test.

Note there was no significant change from baseline following vehicle treatment.

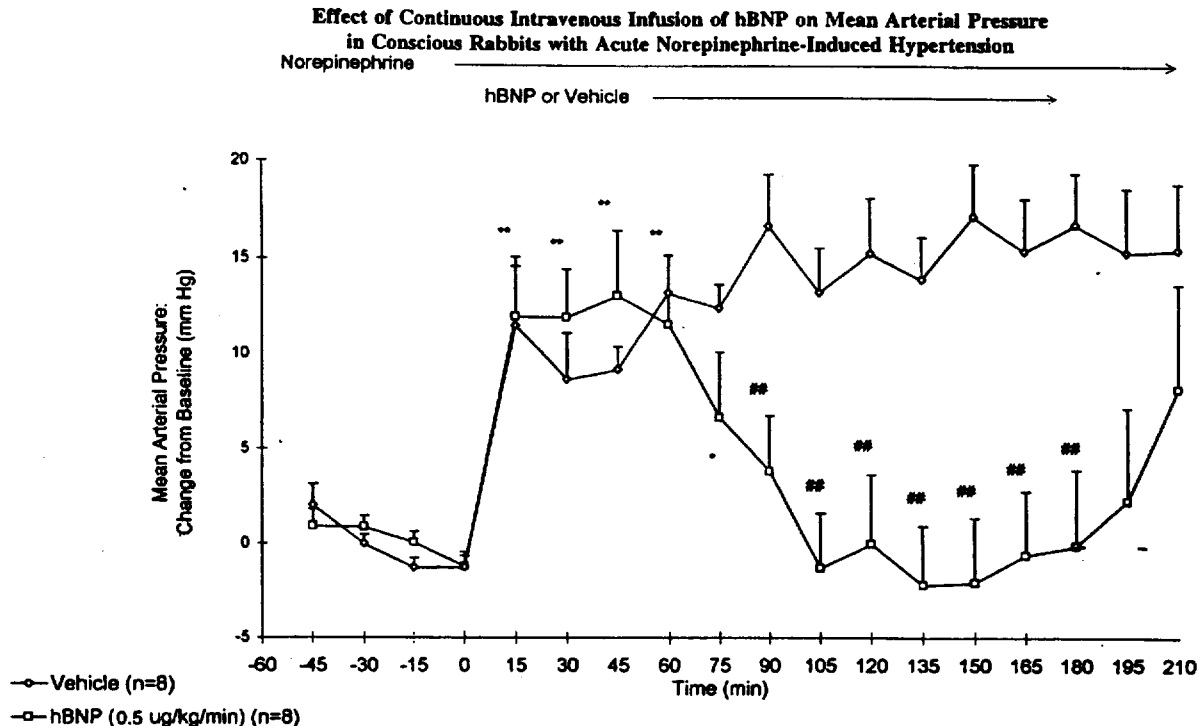
Standard error of the mean is indicated.

Arrow indicates vehicle or hBNP treatment.

In rabbits pretreated with norepinephrine, hBNP decreased the norepinephrine-induced elevations in blood pressure by 90-100% (Figure 12; Sponsor's Figure 6). Blood pressure at the 0.5 $\mu\text{g/kg/min}$ dose appeared to rise during the last 30 min of infusion. There was no statistically significant difference between the 0.1 and 0.5 $\mu\text{g/kg/min}$ doses of hBNP on blood pressure. hBNP did not reduce blood pressure below baseline values (blood pressure before norepinephrine infusion).

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Figure 12 (Sponsor's Figure 6)



*P < 0.05, **P < 0.01 for comparison of values at each timepoint from the hBNP treated groups versus vehicle treatment with Tukey-Kramer multiple comparisons test.

*P < 0.05, **P < 0.01 for comparison of values at each time point in vehicle and hBNP groups versus baseline (t = 0) with Dunnett multiple comparison test. Standard error of the mean is indicated.

Conclusions: Continuous infusion of hBNP at 0.1-0.5 µg/kg/min decreased systolic, diastolic, and mean arterial pressures in normotensive rabbits and in rabbits with norepinephrine-induced hypertension. The effect on blood pressure reached a nadir by about 105 minutes, but there appeared to be a trend for blood pressures to begin to rise thereafter even in the presence of continued drug infusion.

2.9. Cardiovascular and renal actions and pharmacokinetics of Natrecor hBNP administered intravenously and subcutaneously to rabbits (Study No. 00123; Vol. 14 pp. 2-24):

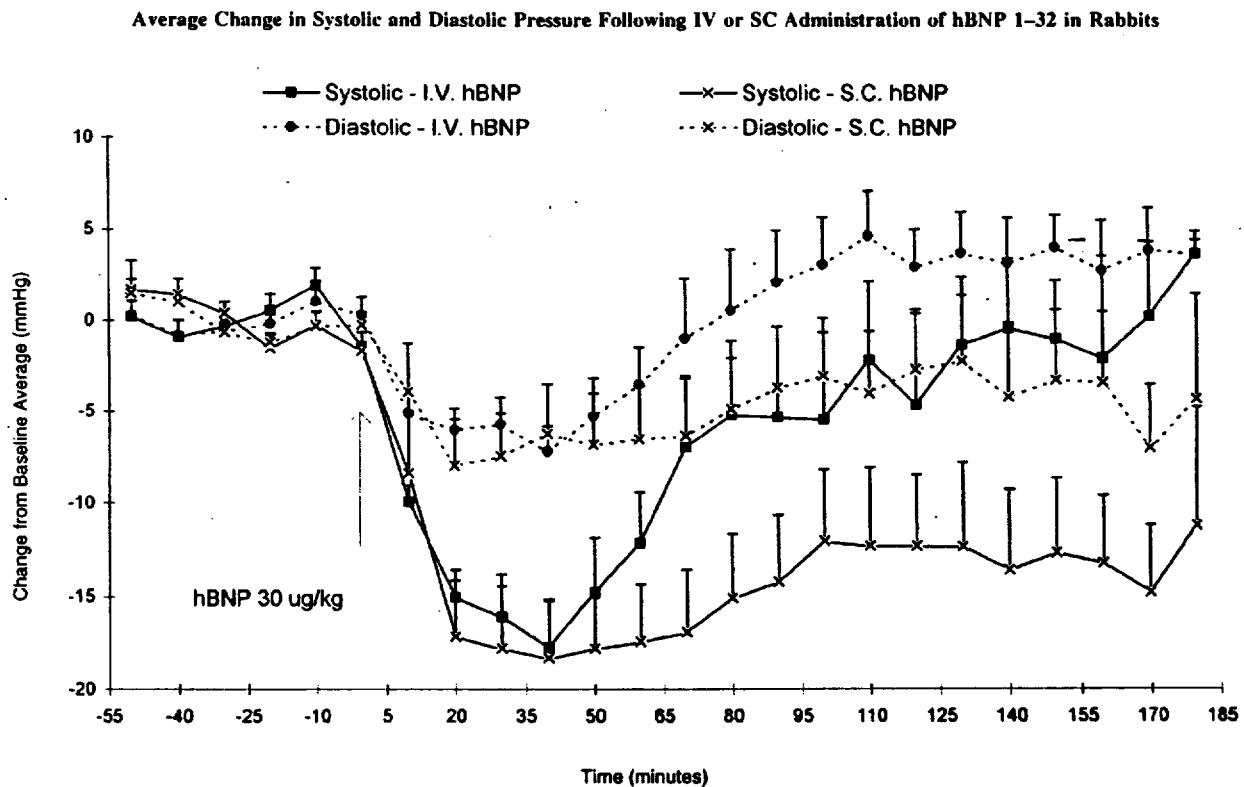
Purpose: This study examined the cardiovascular effects (heart rate and systolic and diastolic blood pressure), renal effects (urine volume, urine sodium, and urine potassium), and pharmacokinetics (AUC, C_{max}, T_{max}, T_{1/2}, V_d, and CL) of hBNP after bolus intravenous (i.v.) and subcutaneous (s.c.) administration in anesthetized rabbits.

Methods: Male New Zealand White rabbits were anesthetized with pentobarbital. The femoral artery was catheterized for measurement of cardiovascular parameters (heart rate and systolic and diastolic blood pressure). Urine was collected with a bladder catheter. Rabbits were given a single bolus intravenous or subcutaneous injection of hBNP at 30 µg/kg. Blood was

collected for determination of plasma hBNP concentrations by an antigen displacement, enzyme-linked immunoassay.

Results: Cardiovascular effects: Both intravenous and subcutaneous administration of hBNP resulted in a decrease in systolic and diastolic blood pressures (Figure 13; Sponsor's Figure 2). Heart rates tended to increase following both routes of administration. The drop in systolic blood pressure was more prolonged following subcutaneous administration.

Figure 13 (Sponsor's Figure 2)

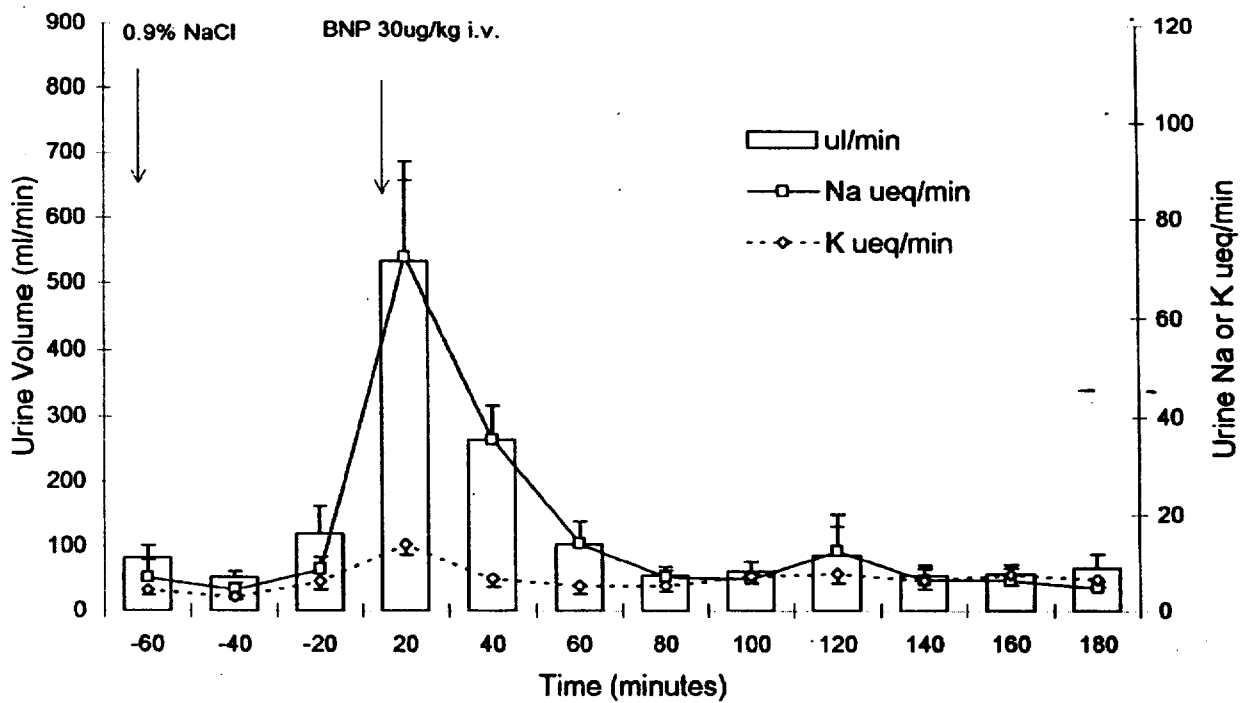


Renal effects: Urine volume and urine sodium were increased after both intravenous (Figure 14A; Sponsor's Figure 4) and subcutaneous (Figure 14B; Sponsor's Figure 5) administration. There was a minimal increase in potassium (kaliuresis). The increases occurred mostly during the first two or three 20 min collection periods. The increases in urine volume and urine sodium over baseline during the 100 min after intravenous and subcutaneous administration of hBNP are shown in Table 1.

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Figure 14A (Sponsor's Figure 4)

Average Renal Response to hBNP 1-32 (30 μ g/kg IV) in Rabbits (n=9)



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Figure 14B (Sponsor's Figure 5)

Average Renal Response to hBNP 1-32 (30 µg/kg SC) in Rabbits (n=9)

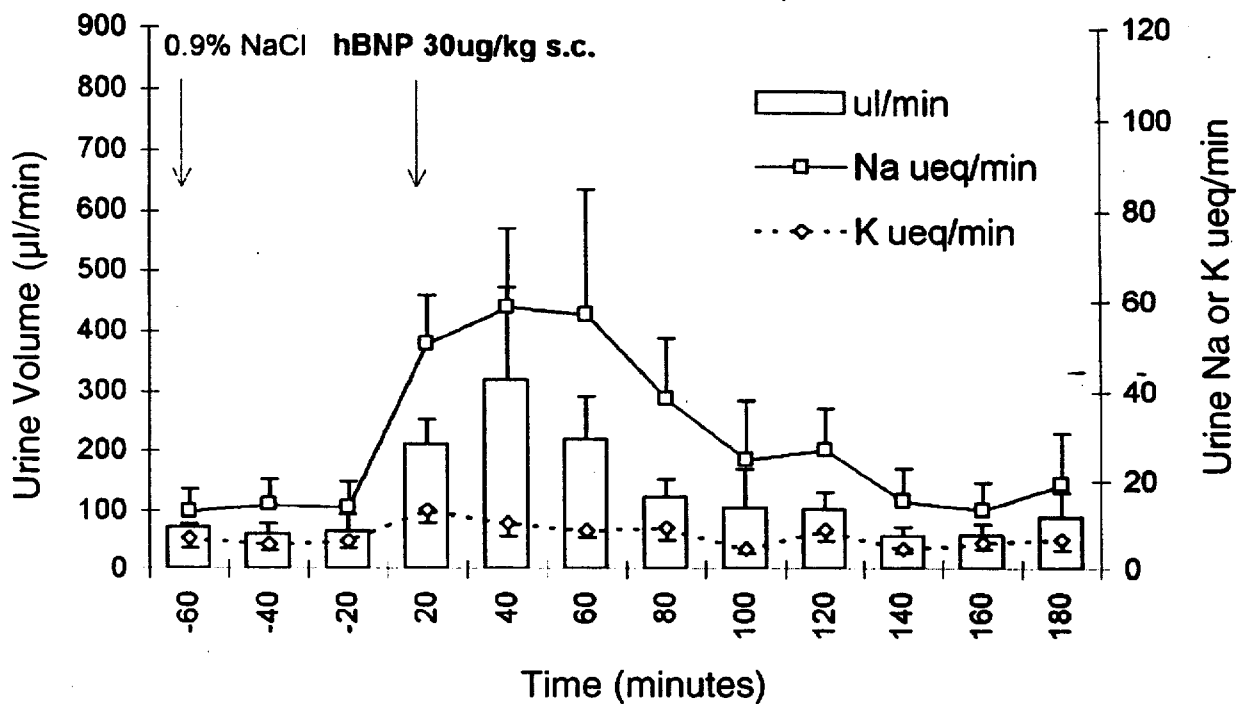


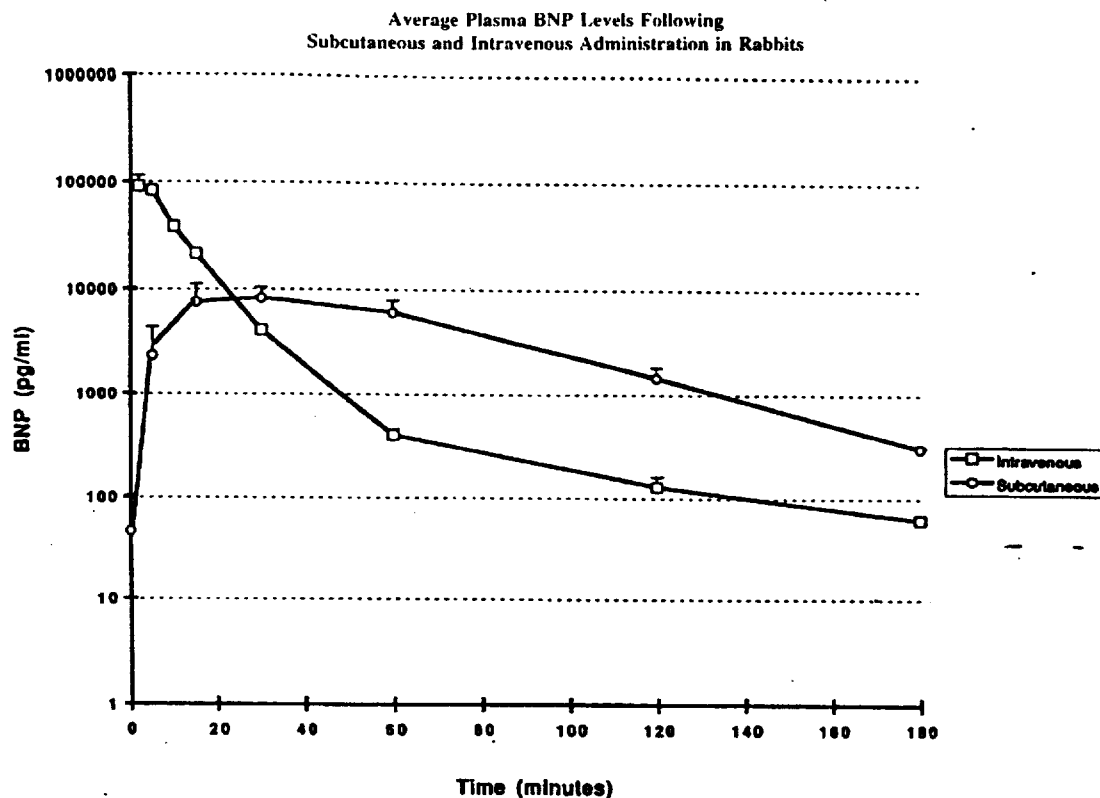
Table 1

Effect of hBNP Administration on Urine Volume and Urine Sodium in Rabbits
(Increase Over Baseline during 100 min after Administration)

Route	Urine Vol. (ml)	Urine Sodium (meq)
IV	9.5 ± 3.3	1.7 ± 0.4
SC	16.2 ± 3.4	3.4 ± 0.8

Pharmacokinetics: Average plasma hBNP levels after i.v. or s.c. administration are shown in Figure 15 (Sponsor's Figure 9).

Figure 15 (Sponsor's Figure 9)



After s.c. administration, plasma hBNP levels were highest (C_{pmax}) at 15-30 min. AUC analysis determined that about 65% of the drug was absorbed by the s.c. route when compared to the i.v. route (Table 2; Sponsor's Table 1).

Table 2 (Sponsor's Table 1)

**Summary of Pharmacokinetics of hBNP
in Rabbits Following Bolus IV or SC Doses**

Route of Delivery	C_{pmax} (ng/mL)	t_{max} (min)	AUC (min • ng/mL)
Intravenous	$92 \pm 15^*$	2*	995*
Subcutaneous	8 ± 2	15-30	656

All values given are with standard deviation.

* These values assume that the 2-minute blood sample, the first sample taken, represented the highest circulating concentration.

Plasma hBNP levels obtained from the intravenous administration were fit to a two-compartment model $T_{1/2a}$ was 5.5 ± 0.9 minutes, $t_{1/2b}$ was 27.4 ± 9.7 minutes, volume of distribution of the central compartment was 0.22 ± 0.05 L/kg, volume of distribution at steady state was 0.28 ± 0.06 L/kg, and volume of distribution by area using method 1 was 1.01 ± 0.35

L/kg and using method 2 was 1.10 ± 0.34 L/kg. Comparison of the pharmacokinetics of hBNP in rabbit, dog, and human is shown in Table 3 (Sponsor's Table 2).

Table 3 (Sponsor's Table 2)

Comparison of hBNP Pharmacokinetics in Rabbit, Canine, and Human

Pharmacokinetic Parameter	Rabbit	Canine	Human*
$t_{1/2 \alpha}$ (min)	5.5 ± 0.9	6.9 ± 1.6	
$t_{1/2 \beta}$ (min)	27.4 ± 9.7	33.2 ± 6.7	
V_c (L/kg)	0.22 ± 0.05	0.24 ± 0.02	
$V_{d_{ss}}$ (L/kg)	0.28 ± 0.06	0.473 ± 0.05	
CL (L/hr/kg)	1.54 ± 0.20	1.38 ± 0.07	

* 20 μ g/kg/dose

Conclusions: hBNP administration to rabbits by either the intravenous or subcutaneous routes resulted in decreased systolic and diastolic blood pressures (hypotension), increased urine volume (diuresis), and increased urine sodium excretion (natriuresis). Potassium excretion (kaliuresis) was minimally increased. Subcutaneous administration resulted in prolonged effects, particularly the drop in systolic blood pressure and transiently enhanced renal output (volume and sodium). AUC analysis showed that about 65% of the hBNP given by the s.c. route was absorbed and active when compared to the i.v. route. These results suggest that mechanisms that may prolong drug clearance or increase the deposition of drug into tissue stores may result in undesirable prolongation of the pharmacodynamic effects.

2.10. Pharmacology and pharmacokinetics of recombinant and synthetic hBNP (Study No. 00184; Vol. 14 pp. 25-71):

Purpose: This study compared the pharmacodynamics and pharmacokinetics of recombinant hBNP (rec-hBNP) and synthetic hBNP (syn-hBNP) *in vitro* (cultured cells) and *in vivo* (rabbits).

Methods: In vitro: Chinese hamster ovary cells (CHO GCA 5A1) expressing the GC-A receptor were incubated with either rec-hBNP or syn-hBNP at concentrations up to 3 μ M for 1.5 hours. After incubation, the medium was assayed for cyclic GMP using an enzyme-linked immunoassay. Potency was calculated as the concentration required to achieve half maximal stimulation of cGMP release (500 pg/10⁶ cells).

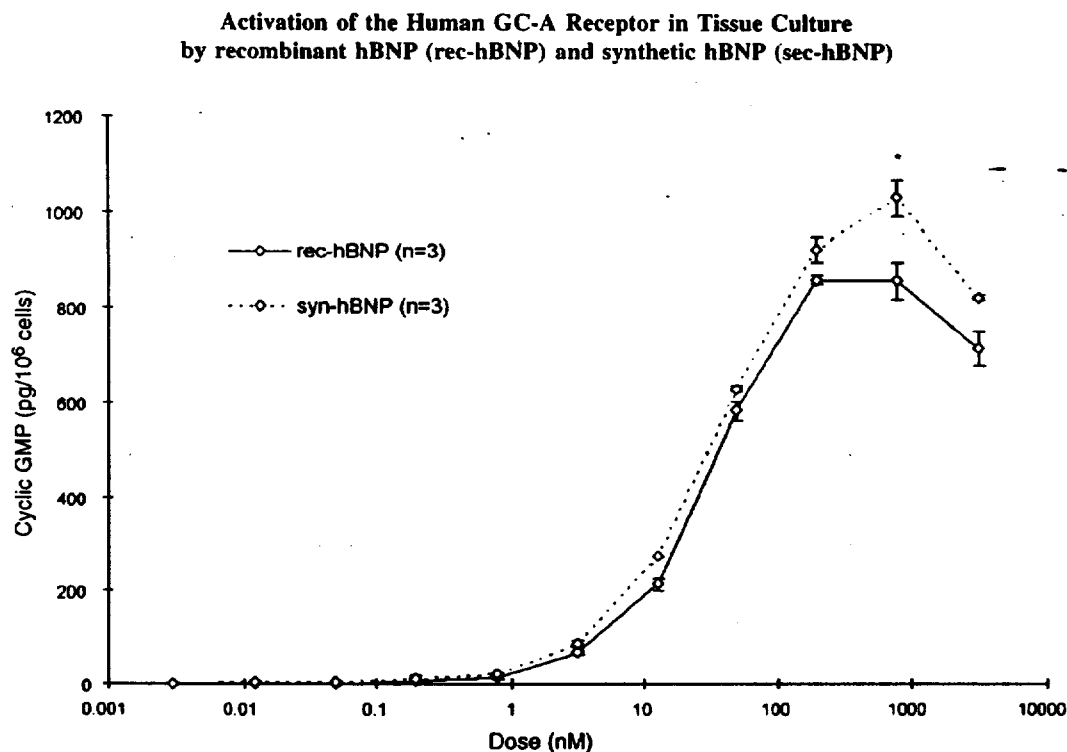
In vivo: Anesthetized male New Zealand White rabbits were catheterized for collection of cardiovascular data (heart rate and systolic and diastolic blood pressure). Urine was collected from a catheter at 20 min intervals. Rec-hBNP or syn-hBNP was given as a single bolus i.v. injection in increasing doses at 1, 3, and 10 μ g/kg.

Conscious rabbits were given either rec-hBNP or syn-hBNP i.v. by either bolus administration or continuous infusion. Bolus doses were 3, 10, and 30 μ g/kg. For continuous

infusion, escalating doses were given at 50, 100, and 200 ng/kg/min for 60 min at each dose. Blood was taken for up to 90 min after bolus administration and at the end of the continuous infusion period for determination of cGMP and immunoreactive hBNP concentrations.

Results: In vitro: Both rec-hBNP and syn-hBNP induced dose-dependent increases in extracellular cGMP when incubated with CHO GCA 5A1 cells for 1.5 hours (Figure 16; Sponsor's Figure 1). The responses to either rec-hBNP or syn-hBNP were essentially similar, although syn-hBNP had a significantly higher peak response at 1 μ M. The derived potencies of rec-hBNP and syn-hBNP were 36 ± 4 nM and 31 ± 2 nM, respectively.

Figure 16 (Sponsor's Figure 1)

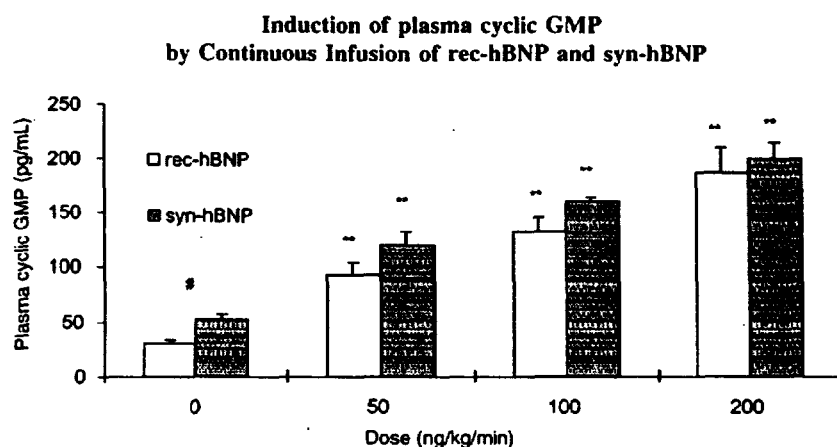


* $P < 0.05$ rec-hBNP vs. syn-hBNP using unpaired two-tailed t-test.

In vivo: In anesthetized rabbits, a single bolus injection of either rec-hBNP or syn-hBNP resulted in transient and dose-dependent increases in urine volume, urine sodium, and urine potassium. The increases in urine volume, urine sodium, and urine potassium were significant at 1 μ g/kg. However, there were no significant differences in renal effects between rec-hBNP and syn-hBNP. Systolic blood pressure, diastolic blood pressure, mean arterial pressure, and pulse pressure were all transiently decreased after a single bolus administration of hBNP. Heart rates increased 20-40 bpm, but there were no significant differences after hBNP treatment when compared to vehicle (saline). There were no significant differences between rec-hBNP and syn-hBNP in any of the cardiovascular parameters.

In conscious rabbits, plasma cGMP increased after a single bolus injection of hBNP. The peak effect occurred less than 10 min after injection. There were no significant differences in plasma cGMP levels between rec-hBNP and syn-hBNP. When given by continuous infusion, cGMP levels showed a dose-dependent increase (Figure 17; Sponsor's Figure 5). Rec-hBNP and syn-hBNP were comparable.

Figure 17 (Sponsor's Figure 5)



** $P < 0.01$ Plasma cyclic GMP values derived from each dose of rec-hBNP and syn-hBNP were compared with pretreatment values by analysis of variance employing Dunnett multiple comparison test.

$P < 0.05$ Plasma cyclic GMP values derived from pretreatment samples were compared using an unpaired, two-tailed student t-test.

In conscious rabbits receiving a bolus dose of either rec-hBNP or syn-hBNP, there were no significant differences in plasma levels of immunoreactive hBNP. Plasma hBNP values derived from animals treated with 10 and 30 $\mu\text{g/kg}$ of rec-hBNP and syn-hBNP were fit to a two compartment model. Plasma hBNP values derived from animals treated with 3 $\mu\text{g/kg}$ of rec hBNP and syn-hBNP were fit to a one compartment model. The half-life values for rec-hBNP and syn-hBNP derived from animals treated with 3 $\mu\text{g/kg}$ of drug were similar (4.7 ± 0.2 minutes and 5.2 ± 0.3 minutes, respectively). Equivalent pharmacokinetic values for half-lives of the α and β phases ($t_{1/2\alpha}$, $t_{1/2\beta}$), micro-rate constants (k_{12} , k_{21} , and k_{13}), volumes of distribution (V_c , V_{dss} , and V_d area), clearance, and area under the plasma concentration-time curve were obtained for rec-hBNP and syn-hBNP (Table 4; Sponsor's Table 14). During a continuous infusion dose escalation protocol, steady-state plasma hBNP concentrations derived for both recombinant and synthetic hBNP dosing groups were equivalent for each of the three doses tested (Figure 18; Sponsor's Figure 18).

Table 4 (Sponsor's Table 14)

Pharmacokinetics of rec-hBNP and syn-hBNP

	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	k_{12} (min ⁻¹)	k_{21} (min ⁻¹)	k_{13} (min ⁻¹)	V_c (L/kg)	V_{dss} (L/kg)	CL (L/min-kg)	V_{darea} (L/kg)	AUC ₍₀₋₉₀₎ (ng-min/mL)
30 µg/kg hBNP										
<u>rec-hBNP</u>										
Mean	4.2	15.4	0.013	0.050	0.209	0.142	0.092	0.015	0.351	1262
Std Dev	1.4	2.9	0.004	0.010	0.022	0.123	0.022	0.004	0.154	396
<u>syn-hBNP</u>										
Mean	3.5	16.2	0.012	0.046	0.215	0.129	0.131	0.022	0.508	858
Std Dev	1.2	1.9	0.001	0.005	0.015	0.067	0.030	0.004	0.064	146
10 µg/kg hBNP										
<u>rec-hBNP</u>										
Mean	3.7	15.0	0.019	0.053	0.186	0.087	0.117	0.016	0.344	480
Std Dev	1.0	0.5	0.007	0.002	0.017	0.025	0.032	0.004	0.089	101
<u>syn-hBNP</u>										
Mean	3.3	15.5	0.015	0.051	0.189	0.107	0.140	0.020	0.447	348
Std Dev	0.4	2.7	0.002	0.009	0.027	0.019	0.022	0.002	0.089	46

Plasma hBNP values were fitted to a two compartment model assuming drug concentrations decline biexponentially as the sum of two first-order processes as described by the formula:

$$Ct = A \exp(-\alpha \cdot t) + B \exp(-\beta \cdot t)$$

Values for $t_{1/2\alpha}$ and $t_{1/2\beta}$ were calculated from $0.693/\alpha$ and $0.693/\beta$, respectively. Micro rate constants, k_{12} , k_{21} and k_{13} , were calculated for each animal using the formulas:

$$k_{12} = A - B - (\beta - \alpha) / [(A+B) - (A - \beta + B - \alpha)] \quad k_{21} = (A - \beta + B - \alpha) / A + B \quad k_{13} = A + B / (A/\alpha + B/\beta)$$

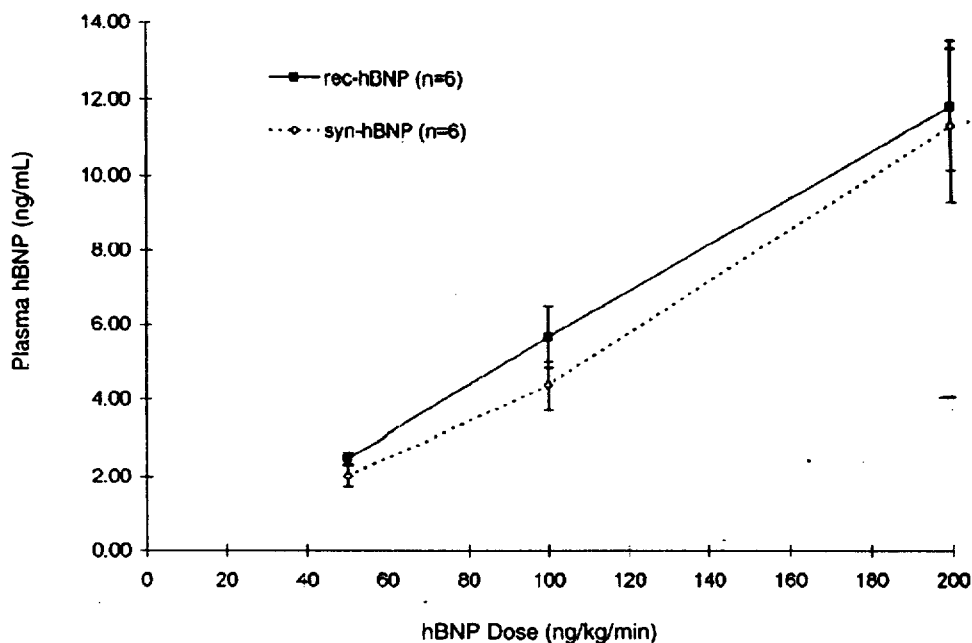
Pharmacokinetic parameters, clearance (CL), volume of distribution of the central compartment (V_c), volume of distribution at steady-state (V_{dss}), and volume of distribution by area (V_{darea}) were derived using the formulas:

$$V_c = \text{Dose} / A + B \quad V_{dss} = [k_{12} + k_{21}] / K_{21} - V_c \quad V_{darea} = (k_{13} - V_c) / \beta \quad CL = k_{13} - V_c$$

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Figure 18 (Sponsor's Figure 18)

**Steady-state Plasma hBNP Concentration
Following IV Continuous Infusion of recombinant and synthetic hBNP**



Conclusions: Both rec-hBNP and syn-hBNP induced comparable dose-dependent increases in extracellular cGMP when incubated with cells expressing the GC-A receptor. In rabbits, a single bolus injection of either rec-hBNP or syn-hBNP resulted in transient and dose-dependent increases in urine volume, urine sodium, and urine potassium. The activities of the two forms were comparable. When given by continuous infusion, both forms caused a comparable dose-dependent increase in plasma cGMP levels. During a continuous infusion dose escalation protocol, pharmacokinetic values and steady-state plasma hBNP concentrations derived for both recombinant and synthetic hBNP dosing groups were equivalent. The half-life values for rec-hBNP and syn-hBNP derived from animals treated with 3 μ g/kg of drug were similar (4.7 ± 0.2 minutes and 5.2 ± 0.3 minutes, respectively).

2.11. Evaluation of the effects of human B-type natriuretic peptide on the performance of isolated, Langendorff-perfused rabbit hearts (Study No. 00280; Vol. 14 pp. 72-94):

Purpose: Effects of hBNP on cardiac performance were examined by monitoring changes in the rate of contraction, coronary flow, and ventricular contractile function in isolated Langendorff-perfused rabbit hearts.

Methods: Male New Zealand White rabbits were anesthetized and the hearts removed and mounted via the aortic root to a cannula. Hearts were perfused retrograde with physiological salt solution (PSS) at 37° C. A catheter was placed into the left ventricle to measure left

ventricular pressure and rate of contraction. In addition, the rates at which left ventricular pressure increased and decreased during each contraction cycle, i.e., $+dP/dt_{max}$ and $-dP/dt_{max}$, were also determined. The pulmonary artery was then cannulated to collect coronary effluent for measurements of coronary flow. Increasing concentrations of hBNP or dobutamine were infused into the perfusate above the heart at a rate of 0.126 mL/min using an infusion pump at the following rates: 0.01 $\mu\text{g}/\text{min}$, 0.1 $\mu\text{g}/\text{min}$, 1.0 $\mu\text{g}/\text{min}$, 10 $\mu\text{g}/\text{min}$, and 100 $\mu\text{g}/\text{min}$. Since differences were observed in coronary flow between the hearts evaluated, the infusion rates employed could not be directly converted to absolute concentrations. However, the infusion rates were approximated to be: 0.0003 $\mu\text{g}/\text{mL}$, 0.003 $\mu\text{g}/\text{mL}$, 0.03 $\mu\text{g}/\text{mL}$, 0.3 $\mu\text{g}/\text{mL}$, and 3.0 $\mu\text{g}/\text{mL}$. Measurements were compared to those obtained with vehicle (PSS).

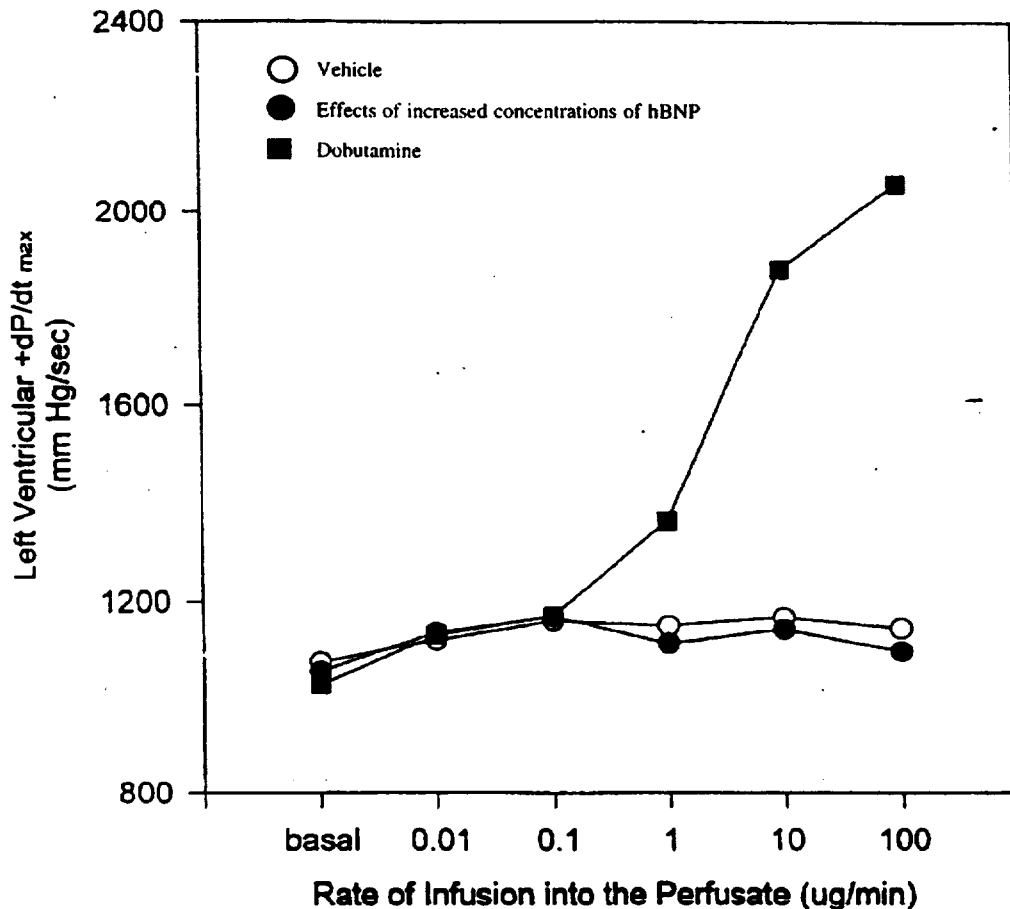
Results: Perfusion of isolated rabbit hearts with hBNP did not appear to produce any remarkable effects on cardiovascular performance when compared with results obtained in the vehicle-treated hearts. Infusion with dobutamine produced large increases in $+dP/dt_{max}$, $-dP/dt_{max}$, and in the rate of contraction. Effects of hBNP and dobutamine on cardiac contractile function ($+dP/dt_{max}$) is shown in Figure 19 (Sponsor's Figure 1).

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Figure 19 (Sponsor's Figure 1)

Effects of hBNP and Dobutamine on Cardiac Contractile Function



Conclusions: It was concluded that perfusion of isolated rabbit hearts with hBNP at rates ranging from 0.01 $\mu\text{g}/\text{min}$ to 100 $\mu\text{g}/\text{min}$ did not appear to produce any direct effects on cardiovascular performance when compared to effects seen with vehicle.

2.12. Assessment of inotropic effects of human B-type natriuretic peptide in explanted human heart tissue (Study No. 00282, Vol. 14 pp. 95-105):

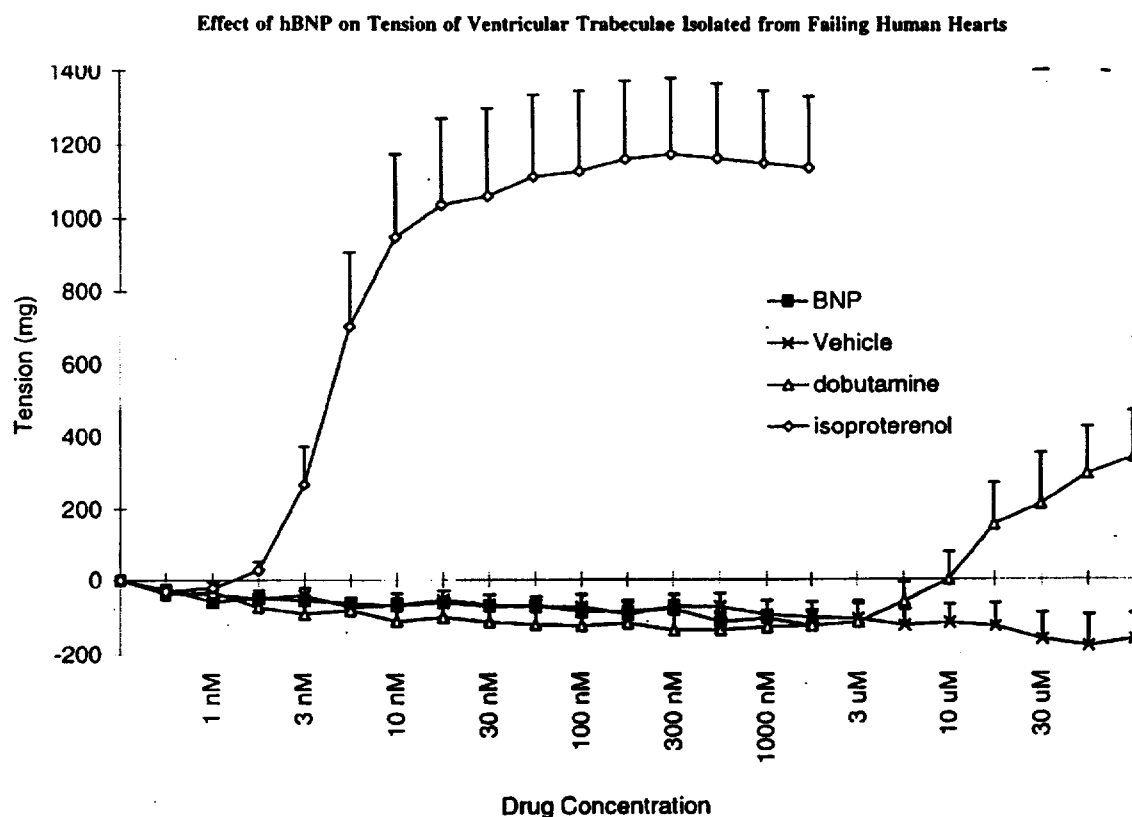
Purpose: The previous study showed that hBNP did not effect cardiac contractility in an isolated rabbit Langendorff-perfused heart preparation. The purpose of this study was to assess whether hBNP exerted a direct inotropic effect on explanted human heart tissue.

Methods: Human failing hearts were obtained from transplant recipients. Individual trabeculae were isolated from the wall of the right ventricle and placed in muscle bath chambers at 37°C. Resting tension was set at the length at which maximum isometric tension developed,

and a field current was passed through the bath. Trabeculae were then incubated with vehicle, hBNP, isoproterenol, or dobutamine. hBNP was added in a dose escalating cumulative manner, with the following final concentrations: 1, 3, 10, 30, 100, 300, and 1000 nM. Dobutamine and isoproterenol were each tested individually, in a dose escalating cumulative manner at the following concentrations: 1, 3, 10, 30, 100, 300, 1000 nM, and 3, 10, 30, and 100 μ M. The net tension response was determined by subtracting tension recordings from pre-drug baseline tension. Dose response curves were plotted and an effective concentration for 50% of the maximum effect (EC_{50}) was determined.

Results: hBNP treatment did not significantly change the trabecular tension when compared to vehicle (Figure 20; Sponsor's Figure 1). The EC_{50} for isoproterenol and dobutamine were 6.84×10^{-8} M and 1.94×10^{-5} M, respectively.

Figure 20 (Sponsor's Figure 1)



Conclusions: These results suggest that hBNP did not have a direct effect on human cardiac muscle contractility when compared to two known positive inotropic agents, isoproterenol and dobutamine.

2.13. Characterization of the cardiac electrophysiologic properties of B-type natriuretic peptide in conscious chronically-instrumented dogs (Study No. 00286; Vol. 14 pp. 106-122):

Purpose: This study assessed the effects of hBNP on the sinus node, atrioventricular junction, atrial, and ventricular tissue, and on the inducibility of atrial and ventricular arrhythmias in conscious dogs. The effects of drug treatment on plasma hBNP, plasma cyclic GMP, and mean arterial blood pressure were also measured.

Methods: [Note: These studies were performed in the laboratory of]

Male or female mongrel dogs (6/group of either sex) were anesthetized and intubated. Continuous electrocardiography and pulse oximetry was performed. Three endocardial pacing leads were positioned in the heart, two in the right atrium and one in the right ventricular apex. One catheter was placed in the aorta to record arterial pressure, and one catheter was placed in the inferior vena cava to record venous pressure and to administer fluids and drugs. Dogs were allowed to recover for 5-7 days before administration of drug.

Before administration of drug, bipolar atrial and ventricular electrograms, surface ECG, and arterial and venous pressures were displayed on a monitor and stored on an optical disk for subsequent analysis. Hemodynamic and electrophysiologic measurements were obtained at baseline following 1 hour of observation. Recombinant hBNP was then administered as a continuous infusion, initially at 0.03 $\mu\text{g/kg/min}$ and then at 0.09 $\mu\text{g/kg/min}$. Each dose of hBNP was administered for 1 hour before measurements were repeated and the infusion was continued during measurements. Venous samples were obtained at baseline prior to initiating the hBNP infusion, after 1 hour at the 0.03 $\mu\text{g/kg/min}$ dose and after 1 hour at the 0.09 $\mu\text{g/kg/min}$ dose.

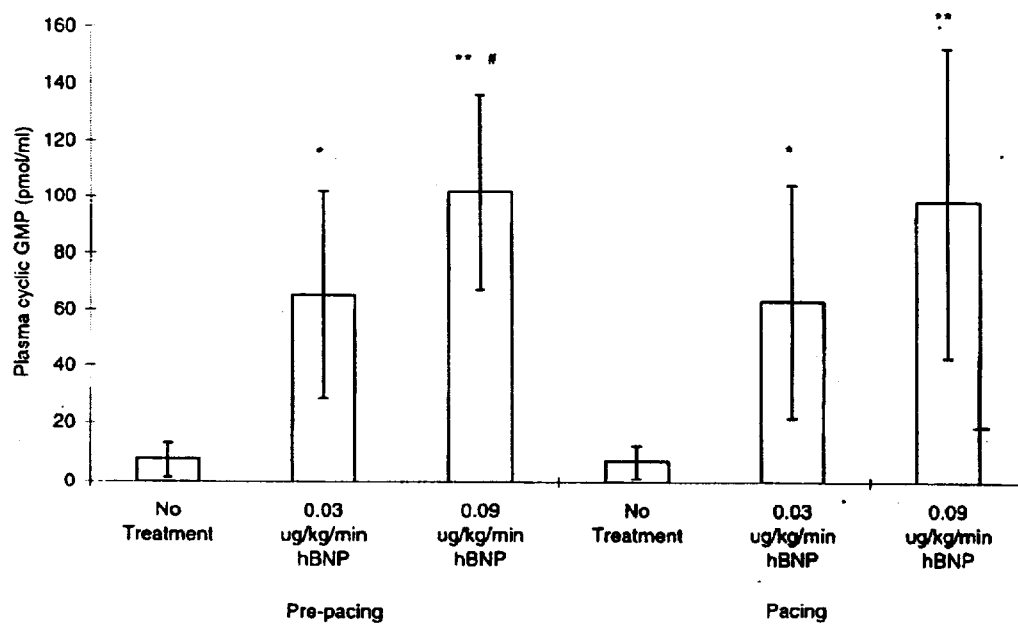
The following electrophysiologic measurements were made: (a) surface electrocardiographic (ECG) intervals (PR, QRS, and QTc), (b) sinus node function, (c) atrial effective refractory period, (d) atrioventricular junction conductance, (e) ventricular effective refractory period, (f) and inducibility of atrial and ventricular arrhythmias. Plasma hBNP levels were determined by an antigen displacement assay. Plasma cyclic GMP levels were determined by radioimmunoassay.

Results: Infusion of dogs with 0.03 and 0.09 $\mu\text{g/kg/min}$ of hBNP resulted in a dose-related increase in plasma cGMP levels (Figure 21; Sponsor's Figure 1) and a dose-related decrease in mean arterial pressure (Figure 22). Heart rate, as measured by the R-R interval, was not increased.

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Figure 21 (Sponsor's Figure 1)

Plasma Cyclic GMP Levels in Dogs Treated With Continuous Intravenous Infusion of hBNP Under Non-Paced and Paced Conditions



* $p < 0.05$

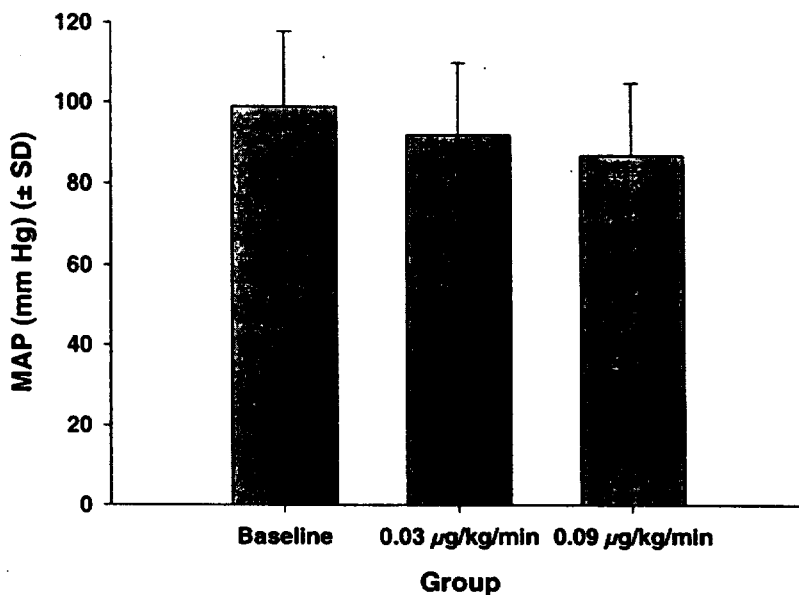
** $p < 0.001$ for hBNP treatment values vs. baseline using repeated measures analysis of variance with Tukey-Kramer multiple comparisons test.

$p < 0.05$ for 0.03 $\mu\text{g/kg/min}$ hBNP treatment vs. 0.09 $\mu\text{g/kg/min}$ hBNP treatment using repeated measures analysis of variance with Tukey-Kramer multiple comparisons test.

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Figure 22

Effect of hBNP on Mean Arterial Pressure (MAP) in Dogs



There were no statistically significant changes in central venous pressure or in any of the ECG parameters measured including surface electrocardiographic intervals (P, PR, and QTc), sinus cycle length (R-R interval), corrected sinus node recovery time, and atrioventricular conduction (Wenckebach cycle length). Effective refractory periods measured at pacing were not altered, and sustained atrial or ventricular arrhythmias were not induced by hBNP infusion.

Conclusions: Infusion of hBNP into conscious dogs at up to 0.09 µg/kg/min for one hour resulted in increased plasma cGMP levels, indicating activation of the biological receptor for hBNP *in vivo*. This was accompanied by a fall in arterial blood pressure which was consistent with the expected hemodynamic effects of hBNP. However, heart rates were not increased as might have been expected in response to reflex sympathetic stimulation or vagal withdrawal, nor were there any changes in any of the electrophysiologic (ECG) parameters measured.

2.14. Differential effects on platelets of two types of cyclic GMP dependent vasodilators: Nitroprusside and B-type natriuretic peptide (Study No. 00272; Vol. 14 pp. 123-136):

Purpose: Two high affinity receptors for hBNP have been described, the guanylyl cyclase-A (GC-A) receptor and the natriuretic peptide clearance (NP-C) receptor. Binding to the GC-A receptor increases intracellular cGMP while binding to the NP-C receptor increases intracellular cAMP. Other studies have shown that both of the vasodilators, hBNP and nitroprusside, increase intracellular concentrations of cGMP, hBNP through binding of the GC-A surface receptor and nitroprusside as a nitric oxide donor. Increased intracellular concentrations of cGMP or cAMP in platelets inhibit their activation and aggregation, an effect which may be undesirable in patients when hemostasis is critical. Human platelets express the NP-C receptor but do not express the GC-A receptor on their surface. This study examined the effects of hBNP and nitroprusside on the activation and aggregation of human platelets.

Methods: Blood was drawn from healthy human donors into sodium citrate anticoagulant and transferred immediately to tubes containing: hBNP (5, 50, 250 nM or 17.6, 176, 880 ng/ml) or sodium nitroprusside (50, 100 μ M). Samples were mixed for 5 min at room temperature. The concentrations of hBNP in the blood of congestive heart failure patients receiving standard treatment doses of hBNP (continuous infusion at 30 ng/kg/min) was stated to be in the range of 2-6 ng/ml.

Drug effects on platelet activation were measured by surface expression of P-selectin in response to adenosine diphosphate (ADP) exposure.

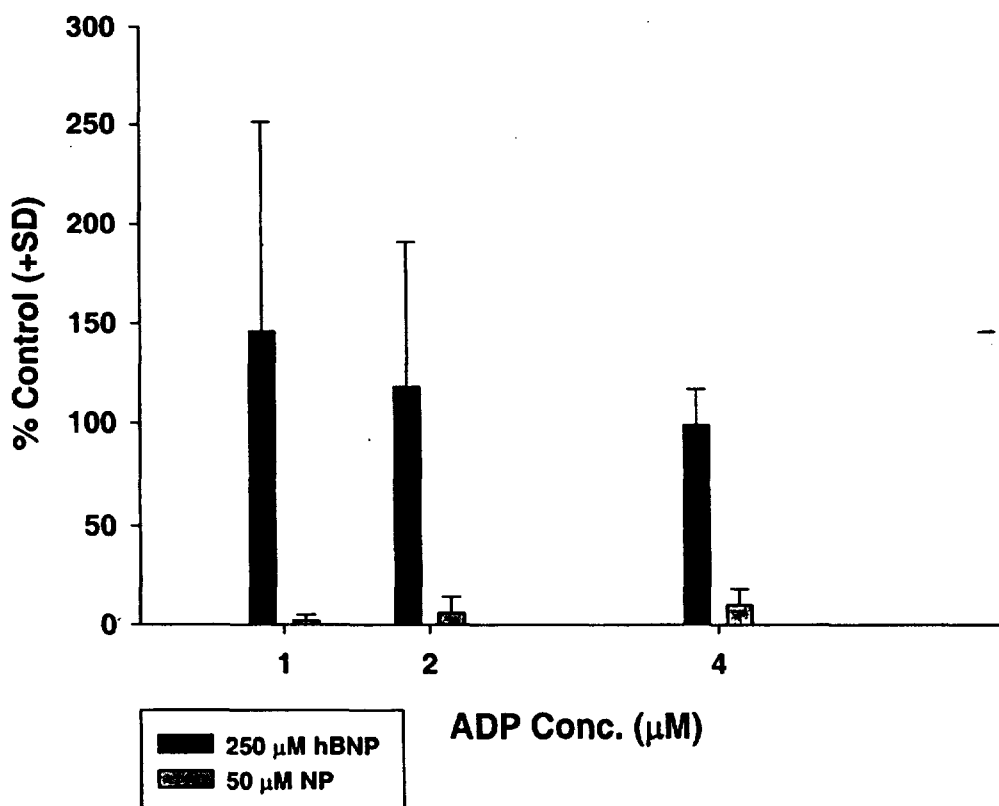
For measurements of drug effects on platelet aggregation, blood samples were first preincubated with test drugs for 5 min. Platelet rich plasma was then prepared by low speed centrifugation of the samples, and aggregation was measured with an aggregometer after initiation of aggregation with either ADP (1-4 μ M) or collagen (5 μ g/ml).

Results: Neither hBNP at up to 250 μ M nor nitroprusside at up to 100 μ M increased P-selectin expression (platelet activation) when tested either alone or in combination with ADP-mediated activation. Platelet aggregation was markedly decreased ($P < 0.001$) by 50 μ M sodium nitroprusside in response to ADP (1-4 μ M) when compared to that seen with ADP alone (Figure 23). However, hBNP at up to 250 μ M did not significantly alter the ADP-induced aggregation of platelets when compared to that seen with ADP alone. Neither compound altered the collagen-induced aggregation of platelets.

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Figure 23

Effect of hBNP (250 μ M) and Nitroprusside (50 μ M)
on ADP-Induced Aggregation of Platelets



Conclusions: hBNP when tested at up to 250 μ M did not induce aggregation or activation of human platelets. In addition, it did not enhance ADP-mediated aggregation and activation. In contrast, sodium nitroprusside markedly decreased platelet aggregation in response to ADP (1-4 μ M) when compared to that seen with ADP alone.

It would have been useful to know the platelet levels of cGMP to determine if the lack of an inhibitory effect by hBNP occurred in spite of increased cGMP levels, and if the enhancing effect of nitroprusside on ADP-mediated platelet aggregation was associated with increased cGMP levels. These data would have helped to determine an association between the expected pharmacologic effect of hBNP on increasing cGMP levels and platelet inhibition.

The sponsor suggested that selection of hBNP as a vasodilator and hBNP's lack of platelet inhibitory function may be useful to patients in whom hemostasis is important.

2.15. Assessment of angiotensin converting enzyme on Natrecor hBNP metabolism *in vitro* and *in vivo* (Study No. 00112; Vol. 14 pp. 137-181):

Purpose: Published studies have shown that the ACE inhibitor captopril increased the half-life of infused porcine BNP in rats from 1.2 min to 7.0 min. Captopril also increased the steady-state concentrations of porcine BNP in rats. The present study examined the effect of purified human ACE on hBNP metabolism *in vitro* and the effect of ACE inhibition with captopril on hBNP metabolism *in vivo* using dogs to determine if hBNP plasma levels may potentially be increased following therapy with ACE inhibitors.

Methods: For the *in vitro* studies, hBNP was incubated at 37° C for 1-60 hours with either human ACE, purified from human cadaver kidneys (laboratory of Dr.), Auriculin (ANP), CNP₁₋₂₂, bradykinin, or angiotensin I.

For the *in vivo* studies, male beagle dogs were given a single i.v. dose of hBNP at 10 µg/kg on Day 1. Blood was taken predose and from 2 to 360 min postdose to determine the half-life of hBNP in dogs. On Day 2, the dogs were given an i.v. loading dose of captopril at 5 mg/kg followed by an infusion at 0.25 mg/kg/hr one hour before administration of 10 g/kg hBNP and continued for 2 hours post-hBNP administration. Blood sampling was the same as on Day 1. Plasma hBNP values were determined using a hBNP-specific immunoassay.

Results: *In vitro*: Preliminary experiments determined that known substrates of ACE, bradykinin, and angiotensin I were metabolized to >97% within one hour of incubation.

degraded. This indicated that hBNP was more resistant to NEP24.11-mediated degradation than ANP.

In vivo: Captopril treatment had no significant effect on the t_{1/2} alpha or beta values for hBNP in dogs (Table 5).

Table 5

hBNP T_{1/2} Values (Mean ± SD) in Dogs
with and without Captopril Treatment

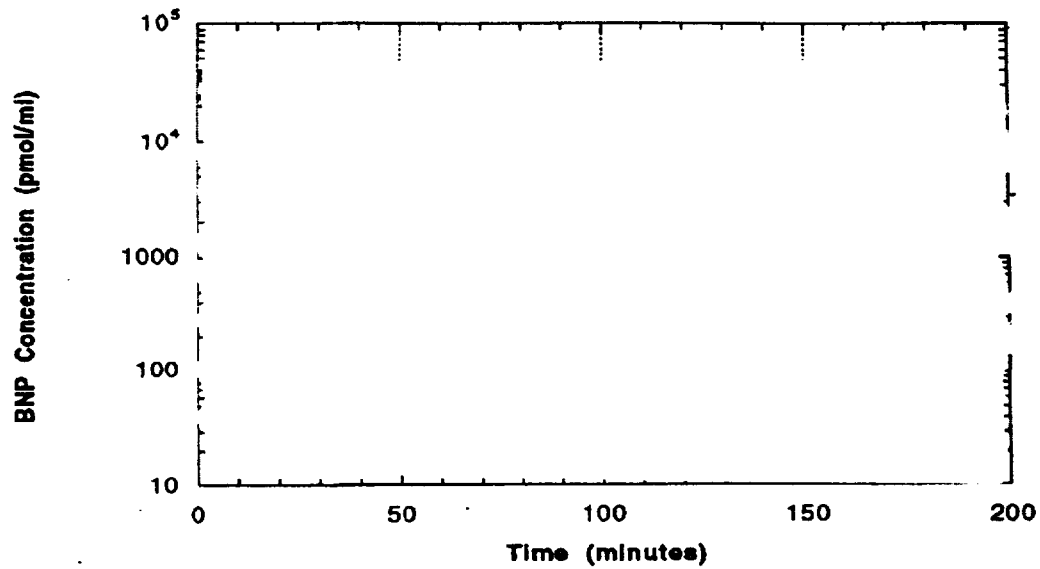
Captopril Treatment	T _{1/2} alpha	T _{1/2} beta
No	6.9 ± 1.6	33.2 ± 6.7
Yes	8.5 ± 4.1	27.6 ± 4.8

Plasma concentrations of hBNP in dogs appeared to be unaffected by captopril treatment (Figures 24A and 24B: Sponsor's Figures 1 and 2). There were no apparent differences in other

pharmacokinetic parameters (micro rate constants, V_c , $V_{d_{ss}}$, and CL) in dogs treated with or without captopril.

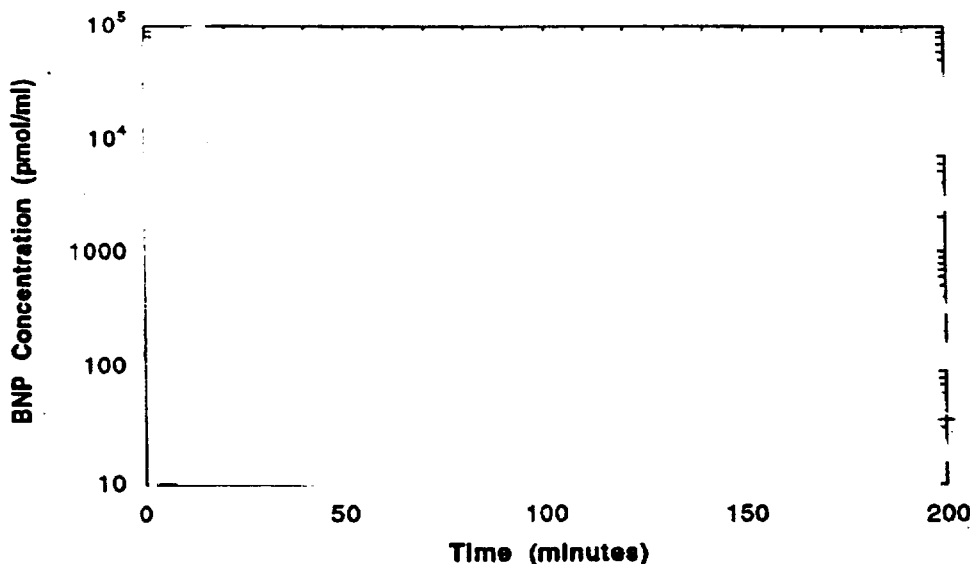
Figure 24A (Sponsor's Figure 1)

Plasma Concentrations of hBNP in Dogs
(No Captopril Treatment)



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Figure 24B (Sponsor's Figure 2)

Plasma Concentrations of hBNP in Dogs
(With Captopril Treatment)

Conclusions: Incubation of hBNP with human ACE *in vitro* did not result in significant ACE-specific degradation of hBNP. *In vivo*, captopril did not change the half-life or other pharmacokinetic parameters of hBNP indicating that hBNP is not a substrate for ACE. This implies that concomitant administration of ACE inhibitors to patients would not alter the pharmacokinetic profile of hBNP.

These results were different from previous results in which ACE inhibition was found to decrease the metabolism of porcine BNP in rats. However, the present study was consistent with published clinical results in which captopril treatment of congestive heart failure patients did not increase the circulating levels of endogenous BNP. The sponsor concluded that there was no significant effect of ACE on hBNP metabolism.

2.16. Effect of heparin on human B-type natriuretic peptide-induced (hBNP) receptor activation *in vitro* and the pharmacokinetics and biological actions of hBNP in rabbits (Study No. 00239; Vol. 14 pp. 182-223):

Purpose: Because both hBNP and heparin may be used in patients with congestive heart failure and postsurgical hypertension, this study examined whether heparin had any effects on the action of hBNP *in vitro*, and if it had any effects on the action and pharmacokinetics of hBNP *in vivo*.

Methods: *In vitro:* CHO GCA 5A1S cells, a Chinese hamster ovary cell line transfected with the human GC-A receptor, were incubated with rec-hBNP (25 or 100 nM) and heparin (1 to 500 units/ml) for 1.5 hours at 37° C. cGMP released into the medium, a measure of GC-A receptor activation, was measured with an enzyme immunoassay.

In vivo: Male New Zealand White rabbits were administered hBNP and heparin by catheters into separate ears. hBNP was given at 50 ng/kg/min for 120 min. Heparin was given at 120 units/kg/hr one hour after beginning of hBNP infusion and continued for 60 min. Blood was taken from the ear artery periodically for up to 120 min after initiation of hBNP infusion and assayed for cGMP and hBNP. Plasma cGMP levels, an *in vivo* measure of GC-A receptor activation, were determined by radioimmunoassay. Plasma hBNP levels were determined by an antigen displacement assay.

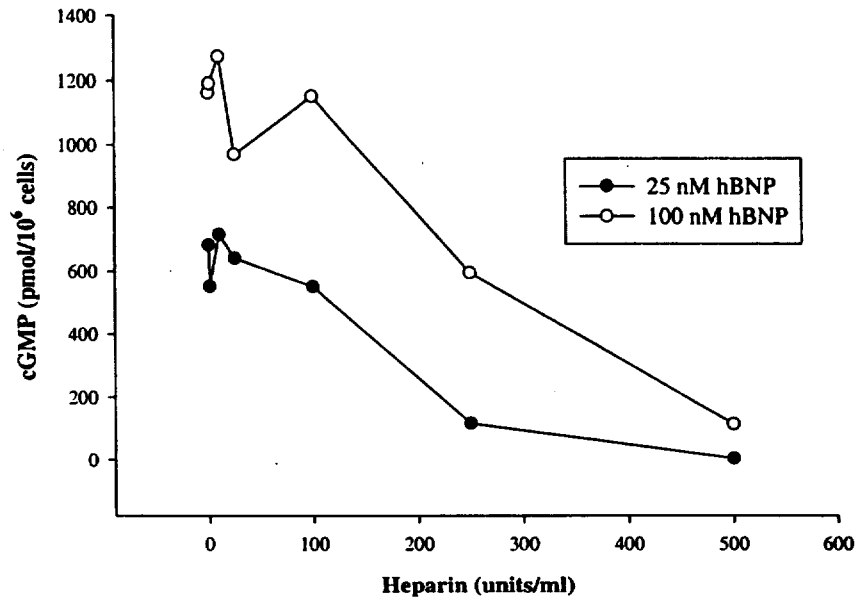
The effect of heparin on hBNP-induced renal and hemodynamic effects in anesthetized rabbits was also studied. In the first protocol (Protocol 2.3), anesthetized rabbits were given saline at 0 min followed by a bolus i.v. dose of 3 µg/kg hBNP at 60 min. At 100 min; heparin was infused at 2 units/kg/min and continued for the duration of the experiment (180 min). At 120 min a second dose of hBNP (3 µg/kg) was given with heparin (20 units/ml; 6.7 units/µg hBNP). In the second protocol (Protocol 2.4), anesthetized rabbits were given saline at 0 min followed by a bolus i.v. dose of hBNP (3 µg/kg) at 60, 135, and 195 min. Heparin was then given at 120 min as a bolus dose of either 10,000 units/kg or 15,000 units/kg (i.e., heparin was given 60 min before and 15 and 75 min after hBNP) followed by continuous infusion at 10,000 or 15,000 units/kg/min. In both protocols, control rabbits received hBNP without heparin. All rabbits were monitored for cardiovascular data and blood pressures. Urine parameters (volume, sodium, and potassium) were monitored throughout.

Results: In vitro: Concentrations of heparin at ≥ 250 units/ml inhibited hBNP-induced cGMP release from CHO cells at both concentrations of hBNP tested (25 and 100 nM) (Figure 25). Heparin concentrations at ≤ 100 units/ml had no effect on hBNP-induced cGMP release. Heparin alone did not induce cGMP release. The sponsor calculated a plasma concentration of heparin after a typical therapeutic dose (a bolus of 5,000 units followed by a maintenance infusion of 1,000 units/hr) to be approximately 2 units/ml when based on a 70 kg individual. If correct, this would imply that heparin would inhibit hBNP at concentrations only that were much greater (>50X) than a typical therapeutic dose.

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Figure 25

Effect of Heparin on hBNP-Induced Release of cGMP from CHO Cells



In vivo: Plasma levels of cGMP after dosing with hBNP rose from a baseline of 46 ± 17 pmol/ml to 100 ± 28 pmol/ml. Infusion of heparin did not significantly change the response seen with hBNP alone (Table 6).

Table 6

Effect of Heparin on Mean Plasma cGMP Levels
Induced by hBNP in Rabbits

Treatment	Mean (pmol/ml) \pm SD
Vehicle	46 ± 17
hBNP	100 ± 28
hBNP + Heparin	112 ± 40

Steady-state plasma levels of hBNP in rabbits were not significantly changed with coinfusion of heparin.

Infusion of hBNP alone (3 μ g/kg) resulted in approximately a 5 to 8X increase in mean urine volume (diuresis), a 4 to 5X increase in mean urine sodium excretion (natriuresis), and a 2 to 3X increase in mean urine potassium excretion (kaliuresis). Systolic blood pressures were

decreased approximately 15 mm Hg. Coinfusion of heparin at either 2 units/kg/min or up to 15,000 units/kg/min did not significantly decrease the renal actions or cardiovascular effects of hBNP. However, in one rabbit the diuretic and natriuretic effect of hBNP was increased about 2X following coinfusion with heparin at 15,000 units/kg/min.

Conclusions: Heparin at concentrations (100 units/ml) up to 50X those found in patients following a typical anticoagulant dose of heparin (2 units/ml) did not inhibit the hBNP-induced release of cGMP from CHO cells *in vitro*. In rabbits, heparin at doses up to 15,000 units/kg, which equals a circulating concentration of 625 units/ml, resulted in no observable decrease on the renal and hemodynamic effects of hBNP. In one rabbit, the diuretic and natriuretic effects of hBNP was increased about 2-fold.

The general lack of an effect of heparin on hBNP-mediated responses in rabbits was inconsistent with a published report (Wei *et al.*, 1987) in which heparin at 700 units/kg inhibited the renal and hemodynamic actions of rat ANP in rats. Possible explanations for the inconsistency were offered including species differences, different heparin binding properties between rat ANP and human BNP, and chemical grade of the heparin used.

3. TOXICOLOGY:

3.1. Acute Toxicity Studies:

3.1.1. Acute intravenous toxicity study with SC-70400 in rats (Study No. 00246; Protocol No. 93-017-70400; Vol. 15 pp. 28-126):

Testing Facility: []
Protocol Number: 93-017-70400
Study Date(s): 7/8/93 to 7/22/93
GLP Compliance: Yes

Purpose: This study assessed the acute toxicity of SC-70400 (hBNP) when given as a single i.v. injection to rats.

Methods: Male and female adult albino Plus rats (5/sex/group; 150-221 gms) were given a single i.v. injection of SC-70400 (lot no. BN006) at 100, 300, 1000, or 3000 µg/kg. Controls received vehicle (5% dextrose). Rats were observed for clinical signs for 14 days. Body weights and food consumption were measured weekly. Two weeks after injection, rats were sacrificed and necropsied by gross examination.

Results: No deaths were reported. No drug-related effects were found on clinical signs, body weight, food consumption, or gross pathology.

Conclusions: A single i.v. injection of SC-70400 to rats at doses up to 3,000 µg/kg produced no evidence of toxicity when assessed by gross examination.

3.1.2. Acute intravenous toxicity study with SC-70400 in Cynomolgus monkeys (Study No. 00247; Protocol No. 93-018-70400; Vol. 15 pp. 127-300):

Testing Facility: []
Protocol Number: 93-018-70400
Study Date(s): 7/7/93 to 7/21/93
GLP Compliance: Yes

Purpose: This study assessed the acute toxicity of SC-70400 (hBNP) when given as a single i.v. injection to cynomolgus monkeys.

Methods: Male and female cynomolgus monkeys (2/sex/group; 2.4-3.1 kg) were given a single i.v. injection of SC-70400 (lot no. BN006) at 60, 180, or 500 µg/kg. Controls received vehicle (5% dextrose). Monkeys were observed daily for clinical signs. Body weights were recorded weekly. Food consumption was measured daily. Physical exams, consisting of heart and respiration rates and rectal body temperatures, were performed before drug injection and two weeks (Day 14) after injection. Blood was collected before injection, on Day 3 after injection, and on Day 14 after injection for clinical pathology (hematology and clinical chemistry). On Day 15, monkeys were sacrificed and a necropsy performed which consisted of a macroscopic (gross) exam. If lesions were found, they were collected and preserved in formalin for microscopic examination.

Results: No deaths were reported. There were no drug-related effects on clinical signs, body weight, food consumption, or physical exam data. The pathology report did not indicate any drug-related effects that could be distinguished from the untreated monkeys. This may reflect the small group size (2/sex/group) and the inter-animal variation. Most macroscopic findings were apparently secondary to parasitic infections.

Conclusions: A single i.v. injection of SC-70400 to cynomolgus monkeys at doses up to 500 µg/kg produced no evidence of toxicity when assessed by gross examination.

3.2. Multiple-Dose Toxicity Studies:

3.2.1. Two-week continuous intravenous infusion toxicity study with SC-70400 in rats (Study No. 00248; Protocol No. 93-019-70400; Vol. 16 pp. 2 to Vol. 18 p. 173):

Testing Facility: []
Protocol Number: 93-019-70400
Study Date(s): 7/13/93 to 8/12/93
GLP Compliance: Yes

Purpose: This study assessed the toxicity of SC-70400 (hBNP) in rats when given as a continuous i.v. infusion for two weeks.

Methods: Male and female adult albino Plus rats (10/sex/group; 232-320 gms for males and 170-219 gms for females) were given SC-70400 (lot nos. BN006, BN008,

and BN005) by continuous i.v. infusion for two weeks at 5, 10, or 20 $\mu\text{g/kg/min}$ using an implanted jugular vein catheter. Controls received vehicle (5% dextrose). Delivery rates were 1 ml/kg/hr. Half the rats (5/sex/group) were treated for two weeks, then the treatment was discontinued, and the rats were observed for toxic effects for at least two weeks posttreatment. All rats were observed for clinical signs twice daily. Body weights and food consumption were measured weekly. Ophthalmic exams were conducted before treatment and at the end of the study. Clinical pathology, consisting of hematology, clinical chemistry, urinalysis, and urine chemistry parameters, were conducted at the end of the treatment and recovery periods. At the end of each study period, rats were sacrificed and a necropsy performed which consisted of a macroscopic exam and weight determination of selected organs. Microscopic examination of 43 tissues was conducted from rats in the control and high dose (20 $\mu\text{g/kg/min}$) groups.

Results: Several rats were sacrificed during the course of the study, three rats due to problems with the catheter and two drug-treated rats due to poor health (one mid-dose female with no notable abnormalities and one high-dose female due to findings consistent with sepsis). Infusion interruptions were common, 3 due to occlusions and 11 due to disconnections. However, all rats received at least 94% of the theoretical dose during the two week treatment period.

Body weights were unchanged, except for some weight gain and increased food consumption in the low and mid-dose females during the first week of recovery. Ophthalmic exams were negative.

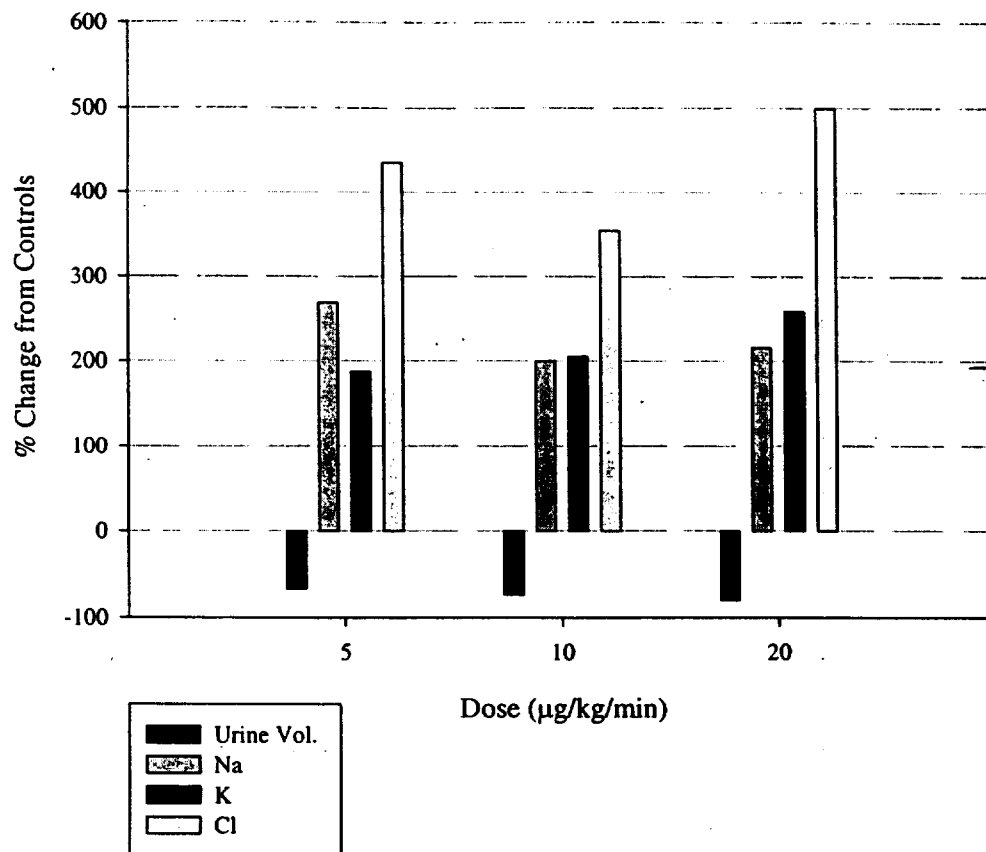
Heart weights were significantly decreased in the ≥ 5 $\mu\text{g/kg/min}$ male and female groups. Kidney weights were increased in the 20 $\mu\text{g/kg/min}$ female group. After a two week recovery period, there were no differences in heart and kidney weights between treated and untreated rats.

Except for lesions at the site of cannula implantation in both treated and untreated rats, microscopic observations were unremarkable with no evidence of systemic toxicity. There were no microscopic changes in the hearts and kidneys to account for the changes in these organ weights.

Clinical pathology results showed many changes that could be considered related to the pharmacological (natriuretic/diuretic) effects of the drug. These included lower serum sodium, chloride, calcium, inorganic phosphorus (males only), and total protein, and higher glucose. Urinalysis in both males and females showed lower urine volume and urine pH and higher urine specific gravity, sodium, potassium, and chloride (Figures 26A and 26B). The magnitude of effects appeared to be similar between the different dose groups (5-20 $\mu\text{g/kg/min}$). These changes were reversed following two weeks of recovery. Although the concentrations of urine electrolytes were higher in the treated groups, the total 16 hour urine excretion of electrolytes was unchanged, indicating that the increased electrolyte concentration was due to concentrated urine rather than increased excretion of electrolytes. The lower urine volume and higher urine specific gravity appeared to be "paradoxical" because of the diuretic properties of SC-70400. This was explained by the sponsor as a homeostatic mechanism (antidiuretic hormone and aldosterone secretion) that were activated following early (within the first few days) diuresis and serum sodium reduction. More frequent urine collections, rather than only at the end of the treatment and recovery periods, could have confirmed this.

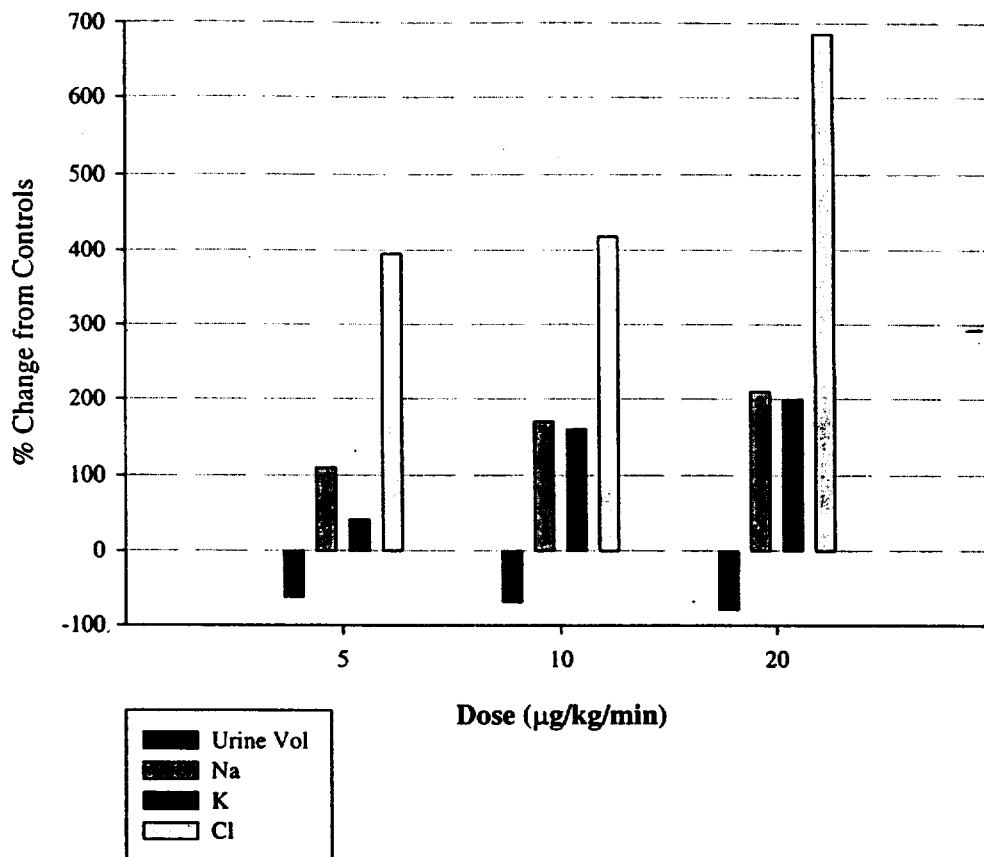
Figure 26A

Effect of SC-70400 on Selected Urinalysis Parameters in Male Rats



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Figure 26B

Effect of SC-70400 on Selected Urinalysis Parameters in Female Rats

Conclusions: Continuous infusion of SC-70400 to rats at doses up to 20 µg/kg/min did not result in histopathological changes indicative of systemic toxicity. Changes in urinalysis parameters, such as decreased urine output and increased urine concentration of electrolytes, were consistent with the known pharmacological properties of the drug as a natriuretic and diuretic agent. No difference in this effect could be distinguished between the low dose of 5 µg/kg/min with the effect seen at the high dose of 20 µg/kg/min. The seemingly paradoxical effect of decreased urine output after two weeks of therapy may have reflected “compensatory homeostatic mechanisms” that were activated early during the course of treatment to conserve fluids and electrolytes. More frequent urine collections could have confirmed this. No clear explanations were offered for the decreased heart and increased kidney weights. However, both organs are sensitive and respond to hemodynamic changes which may occur during the course of

treatment with diuretic and natriuretic compounds. There was no evidence of microscopic changes in these organs to indicate cellular alterations. All alterations in urine, heart and kidney weights were reversible after two weeks of recovery. Pharmacokinetics were not evaluated.

A no-observable-adverse-effect level (NOAEL) could not be determined due to the changes in urinalysis parameters and decreased heart weights seen at the low dose of 5 µg/kg/min. However, these changes were reversible and, except for decreased heart weight, may have been due to the known pharmacologic effects of SC-70400.

3.2.2. Two-week continuous intravenous infusion toxicity study with SC-70400 in monkeys (Study No. 00248; Protocol No. 93-020-70400; Vol. 18 p. 174 to Vol. 20 p. 103):

Testing Facility: {
Protocol Number: 93-020-70400
Study Date(s): 7/27/93 to 8/26/93
GLP Compliance: Yes

Purpose: This study assessed the toxicity of SC-70400 (hBNP) in cynomolgus monkeys when given as a continuous i.v. infusion for two weeks.

Methods: Male and female cynomolgus monkeys (4/sex/group; 2.5-3.8 kg) were given SC-70400 (lot no. BN006) by continuous i.v. infusion for two weeks at 0.3, 1.0, or 3.0 µg/kg/min using a cannula implanted into the femoral vein. Controls received vehicle (5% dextrose). Delivery rates were 3 ml/kg/hr. Two monkeys/sex/group were treated for two weeks, the treatment was discontinued, and the monkeys were observed for toxic effects for at least two weeks posttreatment. Monkeys were observed daily for mortality, moribundity, and signs of toxicity. Body weights were recorded weekly. Food consumption was recorded daily. Physical exams (respiration rates and rectal body temperatures) were conducted before treatment, on the first day of treatment, and weekly thereafter. Blood pressure and ECG measurements (leads I, II, II, aVR, aVL, and aVF) were taken before treatment, weekly during treatment, and at the end of recovery. Ophthalmic exams were conducted before treatment, after two weeks of treatment, and after two weeks of recovery.

Hematology, clinical chemistry, urinalysis, and urine chemistry parameters were evaluated before treatment, weekly during treatment, and at the end of recovery. Blood was also collected predose, at 6 hours and 24 hours and on Days 7 and 14 after beginning of infusion for determination of plasma drug levels [Note: The pharmacokinetic data from this study was previously submitted with the IND but not with the NDA. It has since been submitted to the NDA file]. At sacrifice, a necropsy was performed on all animals that consisted of a macroscopic exam, weight determination of 16 organs from all animals, and microscopic examination of 46 tissues from only control and high dose monkeys.

Results: No deaths were reported. All monkeys, including controls, lost weight during the course of the study. When compared to mean body weights at Day -1, treated animals after 14 days of drug infusion lost slightly more weight (5-6%) than vehicle-infused monkeys (1-2%). During the recovery phase, body weights of treated monkeys were similar to controls. There were no drug-related effects during physical examination (respiration rates and rectal

temperatures). Ophthalmic exams were negative and there were no drug-related effects on surface ECG measurements.

Systolic, diastolic, and mean arterial blood pressures were reduced equally at all doses beginning on Day 3 (first time point measured) in both males and females. After two weeks of recovery (Day 29) blood pressures in the high dose groups (3 $\mu\text{g/kg/min}$) were approaching those of controls. Figures 27A and 27B show only the mean arterial pressures (MAP). This was considered a pharmacologic effect and was consistent with the relaxant or vasodilatory activity seen in precontracted cultures of human arterial and venous tissue as described above (2.3; Study No. 00256).

Figure 27A

Effect of SC-70400 on Mean Arterial Pressure (MAP) in Male Monkeys

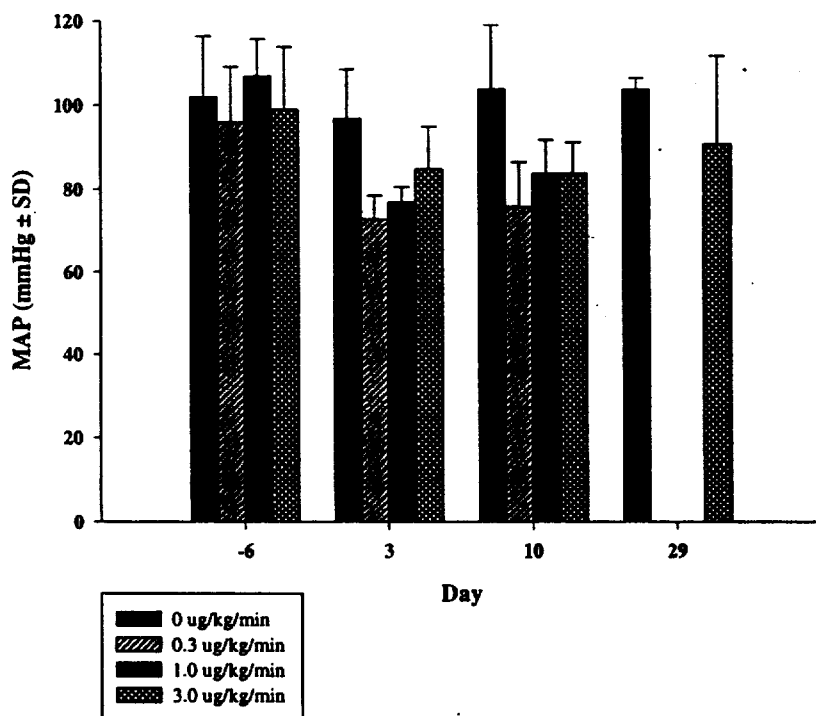
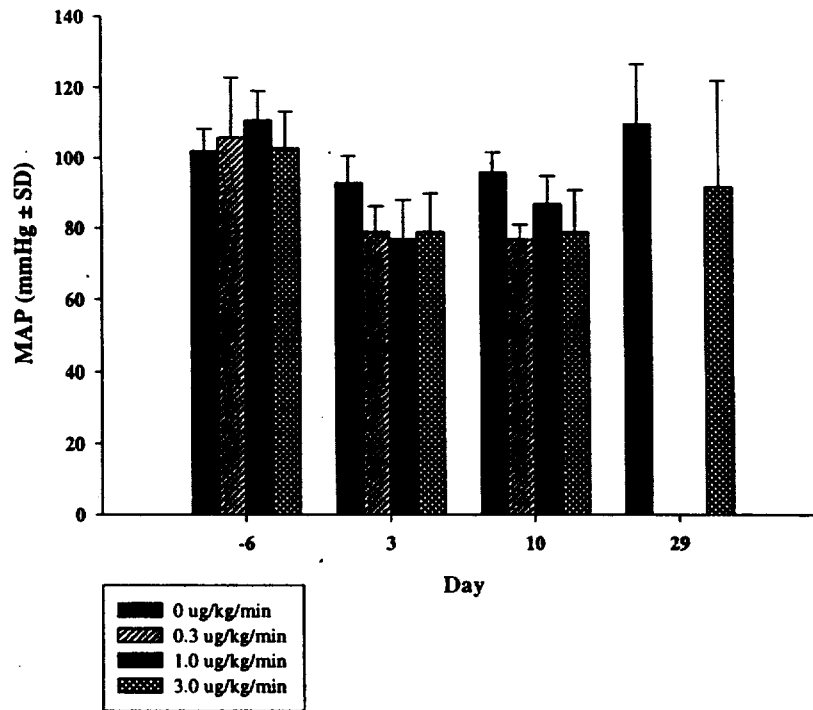


Figure 27B

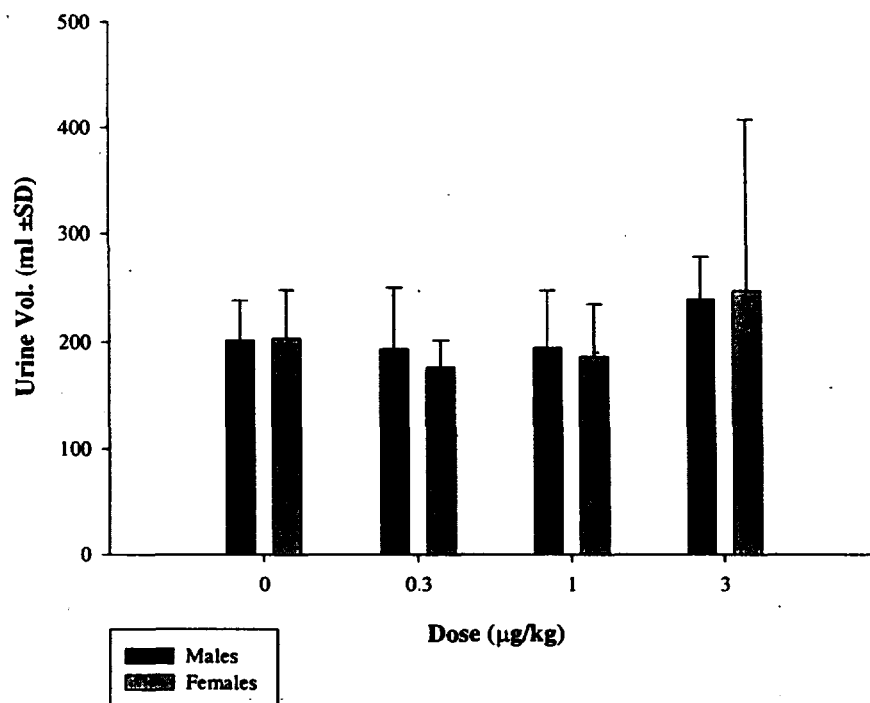
Effect of SC-70400 on Mean Arterial Pressure (MAP) in Female Monkeys

The clinical pathology report stated that there were no obvious test material-related effects on clinical or anatomical pathology results. However, drug treatment did not significantly alter sodium and chloride excretion and urine volume in spite of the drug's known natriuretic and diuretic effects. This may have been due to the large inter-animal variation observed. Mean urine volume (\pm SD) on Day 7 of drug treatment is shown in Figure 28.

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Figure 28

Effect of hBNP Infusion on Urine Volume (Diuresis) in Monkeys
(Day 7)



Macroscopic findings were absent. When compared to controls, heart-to-body weight ratios in males were significantly ($P < 0.05$) reduced at all doses (Figure 29), but the absolute heart weights and heart-to-brain ratios were unchanged. There were no other drug-related effects on or organ weights. Except for the mechanical trauma associated with the indwelling cannula, no microscopic findings related to drug-treatment were found.

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